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Influence of *CYP2B6* genetic variants on plasma and urine concentrations of bupropion and metabolites at steady state

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Abstract

Background—Bupropion, an antidepressant and smoking cessation medication, is metabolized to hydroxybupropion (HB), an active metabolite, primarily by CYP2B6.

Objectives—To compare plasma concentrations of bupropion and metabolites at steady state in healthy volunteers with and without *CYP2B6* genetic variants.

Methods—In a genotype-guided study of 42 healthy subjects we measured plasma and urine concentrations of bupropion and its metabolites, HB, threohydrobupropion (TB) and erythrohydrobupropion (EB) after 7 days of sustained release bupropion dosing.

Results—*CYP2B6*6 and *18* gene variants were associated with approximately 33% reduced concentrations of HB, with no effects on concentrations of bupropion or other metabolites. We could account for 50% of the variation in HB concentrations in a model including genotype and sex.

Conclusions—Since HB is active and steady state concentrations of HB are more than 10 times higher than bupropion, *CYP2B6* variants are likely to affect pharmacological activity. Due to the large individual variation within genotype group, the use of therapeutic drug monitoring for dose optimization may be necessary.

Introduction

Bupropion, a norepinephrine and dopamine uptake inhibitor and nicotinic receptor antagonist, is widely used in the treatment of depression and for smoking cessation. Bupropion is extensively metabolized, with conversion to three main plasma metabolites: hydroxybupropion (HB), threohydrobupropion (TB) and erythrohydrobupropion (EB).[1, 2] The metabolites of bupropion are pharmacologically active: HB is 50%-100% as active and TB and EB 20% as active as bupropion in animal models of inhibition of neurotransmitter uptake or models of depression.[3-6] Bupropion has a half-life ranging from 10 to 20 hrs,

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Conflict of interest statements

Dr Benowitz consults with Pfizer, McNeil and GlaxoSmithKline on smoking cessation medications and has provided paid expert testimony concerning nicotine addiction in litigation against tobacco companies.

Dr. Tyndale has participated in one day advisory meetings for Novartis and McNeil.

and HB has a half-life of about 20 hr, while TB and EB have half-lives of 37 and 33 hours, respectively.[1, 7]

The primary enzyme involved in metabolism of bupropion to hydroxybupropion is the liver enzyme CYP2B6.[8-11] The metabolism of bupropion to TB and EB is primarily by carbonyl reductase, a nonmicrosomal enzyme in the liver and gut. CYP2C19 appears to contribute to oxidation of bupropion to metabolites other than HB.[11] CYP2B6 is polymorphic, with *CYP2B6*6* the most common variant allele [http://www.pharmgkb.org/]. A genotype containing at least one *CYP2B6*6* allele is found in about 25%, 45%, 50% and 70% of Asians, Caucasians, African Americans and Yupik Alaskan Native Peoples, respectively.[12-14] The other common variants include *4 (2-4% allele frequency in Caucasians and Asians, but rare in African Americans) and *5 (10%, 5% and 1% allele frequency in Caucasians, African Americans and Asians, respectively). Relevant to the present study, the *CYP2B6*18* allele is found essentially exclusively in people of African descent, with an allele frequency of 4-7%.

Pharmacokinetic studies in individuals with different genotypes have been performed using single doses of bupropion. Kirchheiner showed that *CYP2B6*4* is associated with faster clearance of bupropion, but that *CYP2B6*6* did not affect bupropion clearance [15]. Loboz [16] reported that one individual who was a homozygote for *CYP2B6 *6* had slower bupropion metabolism than individuals homozygous for the *CYP2B6*1* reference allele. *In vitro* studies support the idea that the *CYP2B6*6* variants are associated with reduced concentrations of functional mRNA, protein and enzymatic activity, thought to be related to aberrant splicing with loss of exons 4 to 6.[17-20] Recombinant expression of CYP2B6.18 in COS-1 cells resulted in marked reduction of expression and reduced bupropion hydroxylase activity. [21] We are aware of no human data on the effect of *CYP2B6*18* on bupropion metabolism.

While single dose studies indicate genetic variation in bupropion metabolism, usual usage for depression or smoking cessation involves prolonged dosing regiments. Thus steady state analyses of the impact of genotype on concentrations of bupropion and metabolites are needed. We conducted a genotype-stratified pharmacokinetic study to compare plasma concentrations of bupropion, HB, TB and EB throughout the day after 7 days of daily dosing with sustained release bupropion in healthy volunteers with and without *CYP2B6*6* variants. We hypothesized that the presence of *CYP2B6*6* alleles would be associated with significant higher steady state plasma concentrations of bupropion and lower plasma concentrations of HB compared to people with reference alleles. We were also able to include a few subjects with the *CYP2B6*18* allele. We hypothesized that *CYP2B6*18* allele would be associated with significant higher steady state plasma concentrations of bupropion and lower plasma concentrations of HB compared to people with reference alleles.

Methods

Subjects

Forty-two healthy subjects were studied, of which 62% were men. Self-declared race included 21 white (14 non-Hispanic, 7 Hispanic), 14 African Americans and 7 Asians. The subjects were selected from a pool of people who had given DNA for genotyping for potential future participation in pharmacokinetic studies. We enriched the subject pool for those with *CYP2B6**6 alleles. Subjects could not be taking regular medications (including oral contraceptives) and could not be current alcohol or illicit drug abusers. Subjects were screened by medical history, physical examination, complete blood count, liver and kidney function and EKG. Individuals with a history of seizures, significant prior head trauma or eating disorders were excluded, as these are risk factors for bupropion-induced seizures.

Subjects were compensated for participation. The study was approved the Institutional Review Boards at the University of California San Francisco and University of Toronto.

Procedures

After screening, subjects signed informed consents and were asked to take one bupropion 150 mg XL (sustained release) tablet in the morning before breakfast daily for 7 days. Seven days was taken as adequate time to reach steady state concentrations of bupropion and metabolites.[1] Subjects were asked to call the research associate daily to confirm that he/she had taken the medication and to give the exact time. On Day 7, subjects were admitted to Clinical Study Unit at San Francisco Hospital Medical Center at about 8 am for a 24 hour admission. Blood samples were collected every 4 hours during the day until 12 midnight then again at 8 am the next day. Bupropion degrades in blood, a process that is temperature and pH dependent.[22] Therefore, blood samples were collected in chilled heparinized tubes and centrifuged in a cold centrifuge. Plasma was then separated into a citric acid tube and frozen until analysis. We found that this procedure for handling blood specimens prevented loss of bupropion that has been seen with other sampling procedures.

Genotyping

Common *CYP2B6* alleles (**4* K262R, **5* R487C, **6* K262R & Q172H, and **9* Q172H) were genotyped using two-step allele-specific PCR assay as previously described.[13, 23]. In addition, new genotyping assays were developed for *CYP2B6*18*. In order to avoid the amplification of *CYP2B7* pseudogene, each assay included a gene-specific amplification followed by an allele specific amplification. The gene-specific amplification primers were forward 5'CCTCTCGGTCTGCCCATCTATAAAC3' and reverse 5'AGGAACCCTGTCTCTGTGTGACT3'. The allele specific amplification primers were forward wild type 5'GGACCCCAGCGCCCCCAA3', forward variant 5'GGACCCCAGCGCCCCCAG3', reverse wild type 5'CGATGTGGGCCAATCACCTGTTCAA3', and reverse variant 5'CGATGTGGGCCAATCACCTGTTCAG3'. The assays were verified by sequencing (ACGT Corporation, Toronto ON, Canada).

Analytical Chemistry

Plasma samples were analyzed for concentrations of bupropion and its metabolites, HB, TB and EB using liquid chromatography – tandem mass spectroscopy, as previously described [24, 25]. The lower limit of quantitation for bupropion was 1 ng/ml. Data on within-run and between-run accuracy and precision are provided in supplemental table 1. Urine samples were assayed with and without glucuronide deconjugation. The deconjugation procedure followed the method of Petsalo, modified with overnight instead of 6 hour incubation.[26] Concentrations of conjugated bupropion or metabolites were determined as the difference between concentrations measured with compared to without deconjugation. Concentrations of bupropion and metabolite standards were unaffected by the deconjugation procedure.

Data Analysis

The main measure of exposure to bupropion and its major metabolites was the area under the plasma concentration-time curve for 24 hours on day 7 (AUC_{24}). Oral clearance of bupropion was also computed as Dose/AUC_{24}, normalized for body weight. Clearance via different metabolic pathways was estimated using the ratio of AUCs for each of the metabolites over bupropion. We also analyzed the maximal concentration (C_{max}) for bupropion and metabolites. Urine data were examined both as absolute concentrations and the molar percentage of each analyte in relation to the sum of bupropion and its metabolites.

Pairwise statistical comparisons were performed by Wilcoxon's tests. Linear regression analysis was used to examine the effects of having zero, one or two copies of *CYP2B6*6* or *CYP2B6*18* allele, race/ethnicity, sex, and BMI on HB AUC and the HB/BUP ratio. To enable better interpretation of the regression results, the PK parameters were not normalized. Normalization by log transformation did not change the significances of the finding. All analyses were performed using Stata 11 (StataCorp, College Station, TX). P values below 0.05 were considered to be statistically significant.

Results

Demographic data and genotype frequencies for the subjects are shown in Table 1. Figure 1 shows the average plasma concentrations of bupropion and metabolites over 24 hours. Steady state HB concentrations were on average more than 10-fold higher than bupropion or EB concentrations, while TB concentrations were intermediate. Pharmacokinetic data for subjects with and without reduced function *CYP2B6* alleles are shown in Table 2. The Cmax, Css (mean steady state concentration) and AUC of HB were significantly lower in subjects with at least one reduced function alleles (i.e either *CYP2B6*6* or **18*) compared to those without, which could also be seen within the *CYP2B6*6* group alone. Consistent with this finding, the ratio of HB/BUP was significantly lower in those with *CYP2B6*6* variants. Other comparisons were not statistically significantly different. Figure 2 shows HB concentrations (AUC) according to *CYP2B6* genotype, sex and race. On average, homozygous and heterozygous *CYP2B6 *6* subjects had lower HB concentrations compared to those without reduced function alleles. Urine bupropion and metabolite excretion data are shown in Table 3. Less than 1% of the daily bupropion dose was excreted unchanged as bupropion. The greatest percentage of the bupropion dose was recovered by TB followed by HB. Bupropion was not appreciably glucuronidated, while glucuronide metabolites accounted for about 75%, 25% and 10% of total excreted HB, EB and TB respectively. Subjects with *CYP2B6* reduced function variants, in particular *CYP2B6*6*, excreted significantly less HB compared to those without reduced function variants. Renal clearances averaged 0.17, 0.03, 0.37 and 0.50 ml/min/kg for bupropion, HB, EB and TB, respectively.

Table 4 presents results of a regression analysis of factors that influence HB AUC and the HB/BUP ratio. The presence of the *CYP2B6*6* allele was associated with significantly reduced HB concentrations and HB/BUP ratio. The *CYP2B6*18* allele was associated with significantly reduced HB concentrations and borderline (p=0.056) lower HB/BUP ratio. Women had a significantly higher HB AUC than men, although this difference was not observed with HB/BUP ratios. Once *CYP2B6*6* and *CYP2B6*18* genotype and sex were accounted for in the regression model, there was no effect of African American race. Asians had a borderline lower level of HB AUC and HB/BUP ratio (P=0.05 and 0.058) which may reflect the presence of additional untested variant alleles.

Discussion

We report several novel findings with respect to the disposition of bupropion in relation to *CYP2B6* genotype. We present the first analysis of steady state concentrations of bupropion and its metabolites in relationship to genotype and race. We confirm findings from previous studies that *CYP2B6*6* does not affect bupropion clearance, but does affect the generation of HB. [15] We present novel data on the functional significance of the *CYP2B6*18* allele in people. We present novel data on renal excretion and clearance of bupropion and metabolites at steady state.

Bupropion is metabolized to HB primarily by CYP2B6, with possible contributions from CYP2E1 and CYP3A4 at high substrate concentrations.[8-11] Bupropion is also

metabolized to hydroxylated metabolites other than HB by CYP2C19.[11] Bupropion metabolism to EB and TB are thought to be catalyzed by carbonyl reductase enzymes.[11]

The *CYP2B6*6* results in a disruption of an exonic splicing enhancer, resulting in a lower amount of functional CYP2B6 mRNA and less protein expression. [17, 18, 20, 23] In addition amino acid changes may contribute to altered function of the protein[27]. In human liver samples, the *CYP2B6*6* variant has been associated with reduced bupropion hydroxylation activities [19, 20], but in human studies this variant did not affect bupropion clearance. [15] Our findings on bupropion blood concentrations during steady state confirm the prior observations with single doses that while it substantially alters HB formation from bupropion, the *CYP2B6*6* variant does not affect bupropion clearance. Presumably this is explained by the multiple metabolic pathways, other than via CYP2B6, through which bupropion is metabolized.

Our results extend the in vitro and in vivo results of other studies that the concentrations of HB are substantially reduced among those with at least on *CYP2B6*6* allele.[15, 16, 19, 25] We found that the HB/BUP plasma AUC ratio decreases in a gene-dose related manner. The magnitude of the effect is substantial with the AUC of HB being decreased by 50% in *CYP2B6*6* homozygotes.

While both bupropion and HB are active, the blood concentrations of HB at steady state are 10-30 times higher than that of bupropion. The pharmacologic effect of a drug is determined by the free drug concentration, which is the product of total blood concentration and % unbound. The % unbound for bupropion is 16% and for HB is 23%[1]. Given the much higher steady state concentration and the greater % unbound, HB is likely to contribute most of the pharmacologic activity of bupropion. The reduction of 50% in HB activity in the *CYP2B6*6* homozygotes is expected to have a substantial impact on the pharmacologic activity and therapeutic efficacy of bupropion; this may alter drug dosing and/or therapeutic response more substantially in some populations compared to others as *CYP2B6*6* homozygotes range from 3% in Asians to over 25% in Yupik peoples.[14]

A limitation of our kinetic study is that we did not measure enantiomers of bupropion or its metabolites. Bupropion is marketed as a racemic mixture. [28] After dosing with a racemic mixture, the blood concentrations of the R- are 3-fold higher than those of S-bupropion[29]. Similarly, HB is enantiomeric, with 2S,3S-HB having greater potency than the 2R,3R- HB at nicotine receptors, while blood concentrations of the 2R,3R are higher than those of 2S,3S after a single dose.[3-5]

Racial differences in the prevalence of the *CYP2B6*6* allele are known: approximately 25% of Asians, 45% of Caucasians, 50% of African Americans have at least one *CYP2B6*6* allele.[12, 13, 25] We are aware of no prior research examining the impact of the *CYP2B6*6* variant on bupropion kinetics in people of different races. In a multiple regression analysis, race had no effect on the HB/BUP ratio when genotype was included in the model, suggesting that the impact of the variant genotypes is similar across races.

Three of our African American subjects had the *CYP2B6*18* variant. While the number of subjects is small, in multiple regression analysis this variant was significantly associated with reduced HB AUC and borderline significantly associated with reduced HB/BUP ratio. The single nucleotide polymorphism that characterizes the *CYP2B6*18* variant causes a change in amino acid sequence which leads to very low CYP2B6 protein expression in mammalian and yeast expression systems[21, 30]. This suggests that *CYP2B6*18* is the causal variant for pharmacokinetic effects, although we cannot exclude the possibility that *CYP2B6*18* has other additional variants in linkage disequilibrium specific to African ancestry. The impact of this allele appears to be greater than the reduction of function found

with *CYP2B6*6*, although with observations in just three subjects this conclusion remains tentative. Based on the allele frequencies of *6 and *18 (6% and 35%, respectively)[25], approximately 16% of African Americans have two copies of either the *6 and/or *18 allele and 49% of African Americans have one copy of either the *6 or *18 allele. This suggests that a large proportion of African Americans have reduced metabolism for CYP2B6 substrates.

We measured bupropion and its major metabolites including glucuronide conjugates in a 24 hour urine at steady state. This allowed us to determine the percent recovery of the daily bupropion dose. Of the daily bupropion dose we could account for only 0.3% as unchanged bupropion and about 10% as the sum of all of measured metabolites in urine. These findings are similar to those seen with administration of C^{14} labeled bupropion.[1] Petsalo has identified 20 metabolites of bupropion in human urine, with the most abundant being m-chlorohippuric acid. [26] Thus, most of the metabolites of bupropion excreted in urine are not the main metabolites measured in plasma.

The most prevalent urine metabolite was TB, followed by HB and EB, with relatively low concentrations of bupropion. As expected, subjects with the *CYP2B6*6* variant excreted significantly less HB compared with those without any reduced function variants. Petsalo identified 8 glucuronide conjugates of bupropion metabolites, including conjugates of HB, TB and EB. We provide the first human data indicating the glucuronidation is greatest for HB, less for TB and EB, and minimal or none for bupropion.

The renal clearances of bupropion and its metabolites were all low, much lower than glomerular filtration rate. The lowest clearances were for bupropion and HB, most likely related at least in part to a high degree of protein binding (84% and 77%, respectively). [1] The renal clearance of TB and EB were higher, consistent with a lower extent of plasma protein binding. Our data on renal clearance of bupropion may have forensic utility, allowing extrapolation from urine concentrations to plasma concentrations at steady state.

With respect to using genotyping to personalize treatment, our data indicate that people who have *CYP2B6*6* and *CYP2B6*18* variants have reduced concentrations of HB and would be expected to have reduced pharmacologic activity from a given dose of bupropion. Consistent with this idea, Zhu et al recently reported that when bupropion is used for smoking cessation in African American smokers, the quit rates were associated with plasma HB but not bupropion concentrations.[25] As expected the HB concentrations were lower in smokers who have reduced function *CYP2B6* variants. It was speculated that smokers with reduced HB formation might need higher doses of bupropion to achieve the same smoking cessation effect, and possibly for its antidepressant effects, compared to those who are normal metabolizers.

Our paper focuses on the effects of the *CYP2B6* genotype on bupropion metabolism. CYP2B6 is also involved in the metabolism of other drugs, such as efavirenz, cyclophosphamide, artemisinin, ketamine, propofol, methadone and selegeline. Studies with efavirenz have shown that the *CYP2B6*6* variant is associated with higher concentrations of parent drug and increased toxicity.[31] This finding of reduced enzymatic activity is consistent with our finding that the *CYP2B6*6* variant is associated with reduced generation of the bupropion metabolite, HB. Whether a person with a *CYP2B6*6* allele requires dose reduction (efavirenz) or dose escalation (possibly bupropion) depends on the extent to which the parent drug vs metabolite mediates pharmacologic activity.

In conclusion we demonstrate for *CYP2B6*6* and provide strong evidence for *CYP2B6*18* that these gene variants are associated with reduced concentrations of the active metabolite HB at steady state, with no effect of concentrations of bupropion or other metabolites. The

effect of the gene variants appears to be independent of race. Our data provide quantitative estimates of gene variant effects, with our model including sex accounting for 50% of the variation in HB concentrations. There is wide variability in HB concentrations within any particular genotype, likely due to additional untested alleles, potential impact of CYP2B6 inducers, and the known impact of sex. Therapeutic drug monitoring should be evaluated as a way to optimize therapeutic dosing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. 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. [PubMed: 12109932]
2. Jefferson JW, Pradko JF, Muir KT. Bupropion for major depressive disorder: Pharmacokinetic and formulation considerations. Clin Ther. 2005; 27:1685–95. [PubMed: 16368442]
3. Damaj MI, Carroll FI, Eaton JB, Navarro HA, Blough BE, Mirza S, et al. Enantioselective effects of hydroxy metabolites of bupropion on behavior and on function of monoamine transporters and nicotinic receptors. Mol Pharmacol. 2004; 66:675–82. [PubMed: 15322260]
4. Damaj MI, Grabus SD, Navarro HA, Vann RE, Warner JA, King LS, et al. Effects of hydroxymetabolites of bupropion on nicotine dependence behavior in mice. J Pharmacol Exp Ther. 2010; 334:1087–95. [PubMed: 20576796]
5. Bondarev ML, Bondareva TS, Young R, Glennon RA. Behavioral and biochemical investigations of bupropion metabolites. Eur J Pharmacol. 2003; 474:85–93. [PubMed: 12909199]
6. Grabus SD, Carroll FI, Damaj MI. Bupropion and its Main Metabolite Reverse Nicotine Chronic Tolerance in the Mouse. Nicotine Tob Res. 2012
7. 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; 21:127–35. [PubMed: 6804243]
8. Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI, et al. CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants. Drug Metab Dispos. 2000; 28:1176–83. [PubMed: 10997936]
9. Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM, et al. Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. Drug Metab Dispos. 2000; 28:1222–30. [PubMed: 10997944]
10. Faucette SR, Hawke RL, Shord SS, Lecluyse EL, Lindley CM. Evaluation of the contribution of cytochrome P450 3A4 to human liver microsomal bupropion hydroxylation. Drug Metab Dispos. 2001; 29:1123–9. [PubMed: 11454731]
11. Chen Y, Liu HF, Liu L, Nguyen K, Jones EB, Fretland AJ. The in vitro metabolism of bupropion revisited: concentration dependent involvement of cytochrome P450 2C19. Xenobiotica. 2010; 40:536–46. [PubMed: 20528624]

12. Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann MH, Schwab M. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. Pharmacogenomics. 2007; 8:743–59. [PubMed: 17638512]

13. 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; 62:635–41. [PubMed: 17223085]

14. Binnington MJ, Zhu AZ, Renner CC, Lanier AP, Hatsukami DK, Benowitz NL, et al. CYP2A6 and CYP2B6 genetic variation and its association with nicotine metabolism in South Western Alaska Native people. Pharmacogenet Genomics. 2012; 22:429–40. [PubMed: 22569203]

15. Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Murdter TE, et al. Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. Pharmacogenetics. 2003; 13:619–26. [PubMed: 14515060]

16. Loboz KK, Gross AS, Williams KM, Liauw WS, Day RO, Blievernicht JK, et al. Cytochrome P450 2B6 activity as measured by bupropion hydroxylation: effect of induction by rifampin and ethnicity. Clin Pharmacol Ther. 2006; 80:75–84. [PubMed: 16815319]

17. 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; 307:906–22. [PubMed: 14551287]

18. Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M, et al. Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. J Pharmacol Exp Ther. 2008; 325:284–92. [PubMed: 18171905]

19. Hesse LM, He P, Krishnaswamy S, Hao Q, Hogan K, von Moltke LL, et al. Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes. Pharmacogenetics. 2004; 14:225–38. [PubMed: 15083067]

20. Desta Z, Saussele T, Ward B, Blievernicht J, Li L, Klein K, et al. Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. Pharmacogenomics. 2007; 8:547–58. [PubMed: 17559344]

21. Klein K, Lang T, Saussele T, Barbosa-Sicard E, Schunck WH, Eichelbaum M, et al. Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. Pharmacogenet Genomics. 2005; 15:861–73. [PubMed: 16272958]

22. Laizure SC, DeVane CL. Stability of bupropion and its major metabolites in human plasma. Ther Drug Monit. 1985; 7:447–50. [PubMed: 3936237]

23. Al Koudsi N, Tyndale RF. Hepatic CYP2B6 is altered by genetic, physiologic, and environmental factors but plays little role in nicotine metabolism. Xenobiotica. 2010; 40:381–92. [PubMed: 20307138]

24. Haas JS, Kaplan CP, Barenboim D, Jacob P 3rd, Benowitz NL. Bupropion in breast milk: an exposure assessment for potential treatment to prevent post-partum tobacco use. Tob Control. 2004; 13:52–6. [PubMed: 14985597]

25. Zhu AZ, Cox LS, Nollen N, Mayo M, Faseru B, Okuyemi KS, et al. CYP2B6 and bupropion's smoking cessation pharmacology: the role of hydroxybupropion. Clin Pharmacol Ther. 2012; 96:771–7. [PubMed: 23149928]

26. Petsalo A, Turpeinen M, Tolonen A. Identification of bupropion urinary metabolites by liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2007; 21:2547–54. [PubMed: 17639567]

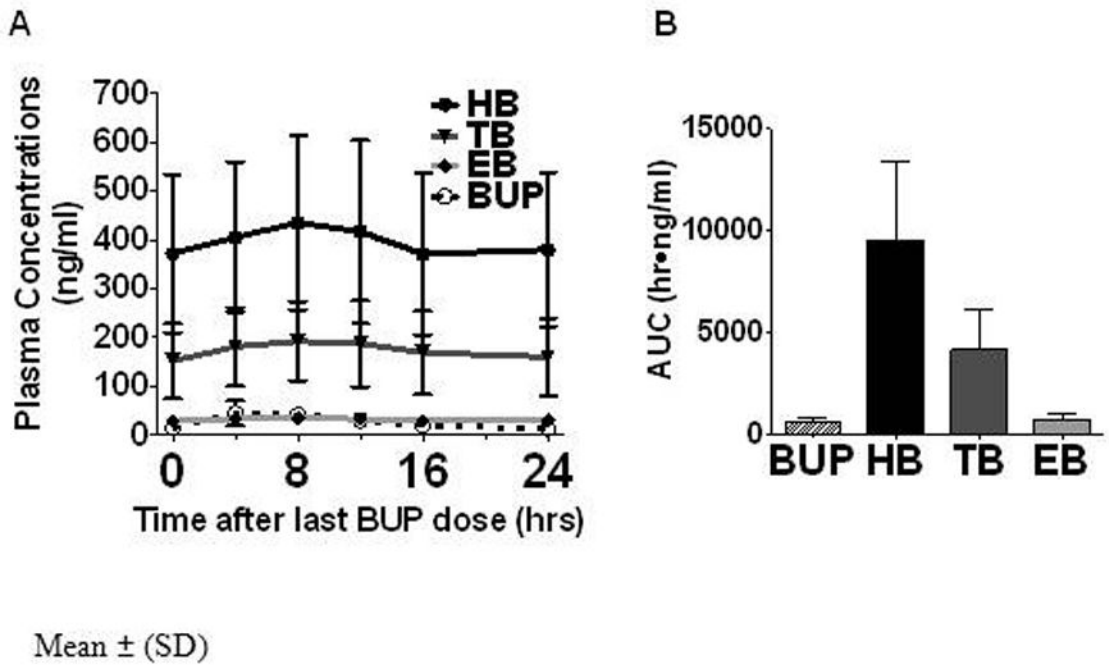
27. Zhang H, Sridar C, Kenaan C, Amunugama H, Ballou DP, Hollenberg PF. Polymorphic variants of cytochrome P450 2B6 (CYP2B6.4-CYP2B6.9) exhibit altered rates of metabolism for bupropion and efavirenz: a charge-reversal mutation in the K139E variant (CYP2B6.8) impairs formation of a functional cytochrome p450-reductase complex. J Pharmacol Exp Ther. 2011; 338:803–9. [PubMed: 21659470]

28. Xu H, Loboz KK, Gross AS, McLachlan AJ. Stereoselective analysis of hydroxybupropion and application to drug interaction studies. Chirality. 2007; 19:163–70. [PubMed: 17167747]

29. Kharasch ED, Mitchell D, Coles R. Stereoselective bupropion hydroxylation as an in vivo phenotypic probe for cytochrome P4502B6 (CYP2B6) activity. J Clin Pharmacol. 2008; 48:464–74. [PubMed: 18287571]

30. Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. Pharmacogenet Genomics. 2006; 16:191–8. [PubMed: 16495778]

31. Ngaimisi E, Mugusi S, Minzi O, Sasi P, Riedel KD, Suda A, et al. Effect of rifampicin and CYP2B6 genotype on long-term efavirenz autoinduction and plasma exposure in HIV patients with or without tuberculosis. Clin Pharmacol Ther. 2011; 90:406–13. [PubMed: 21814190]



Mean ± (SD)

Figure 1.

Panel A: Plasma concentrations of bupropion (BUP) and its metabolites hydroxybupropion (HB), threohydrobupropion (TB) and erythrohydrobupropion (EB) over 24 hours. Mean ± SD

Panel B: Area under the 24 hour plasma concentration-time curve for bupropion and metabolites.

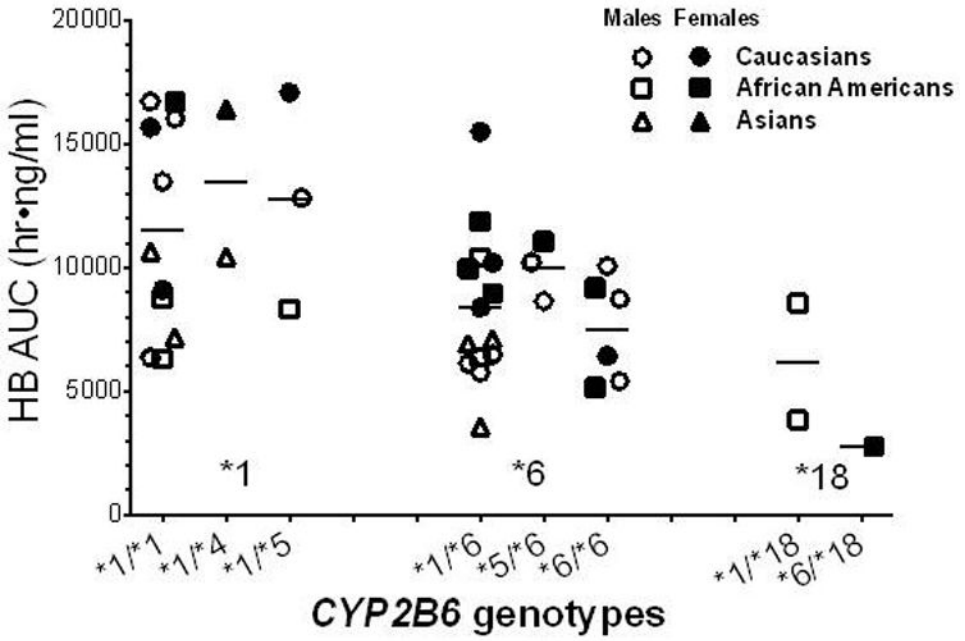


Figure 2.
Area under the 24 hour hydroxybupropion (HB) concentration-time curve in individuals with different *CYP2B6* genotypes, shown by sex and race.

Table 1

Demographics and Genotype Distribution

	White (N=21)	African American (N=14)	Asians (N=7)
Sex (% male)	61.9	50	85.7
Age[a] (y)	29.2 (19,51)	33.1 (22,64)	37.0 (22,60)
Weight (kg)	72.0 (65.9,78.1)	75.6 (66.8,84.4)	80.8 (56.2,105.4)
BMI (kg/m^2)	24.1 (22.3,25.9)	25.6 (23.2,27.9)	29.1 (18.7,39.5)
CYP2B6 Genotype			
*1/*1	6 (29%)	3 (21%)	2 (29%)
*1/*4	-	-	2 (29%)
*1/*5	2 (10%)	1 (7%)	-
*1/*6	6 (28%)	5 (36%)	3 (43%)
*5/*6 or *1/*7	3 (14%)	-	-
*6/*6	4 (19%)	2 (14%)	-
*1/*18	-	2 (14%)	-
*6/*18	-	1 (7%)	-

[a] mean and range

[b] men (95% C.I.)

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Table 2

Pharmacokinetic Parameters for Bupropion and Metabolites (mean, 95% CI)

	All (*N*=42)	No reduced function allele (*N*=16)	With reduced function allele(s) (N=26)	*6 allele (*N*= 23)	*18 allele (*N*= 2)	*6/18 allele (*N*= 1)
BUP						
C_{max} (ng/mL)	58 (52,63)	58 (51,65)	57 (48, 66)	58 (50,67)	59 (-2,121)	33
Css (ng/mL)	28.5 (26.3,30.8)	29.4 (26.6,32.1)	28.0 (24.7,31.4)	28.6 (25.6,31.7)	25.9 (25.6,226.5)	18.8
AUC (hr•ng/mL)	685 (631,739)	705 (639,770)	673 (593, 753)	687 (614,761)	621 (-614,5437)	450
HB						
C_{max} (ng/mL)	464 (406,522)	592 (493,690)	**386[a] (330, 442)**	**405[a] (352,459)**	291 (130,452)	137
Css (ng/mL)	397 (347,447)	501 (410,591)	**333[b] (285,381)**	**349[b] (301,397)**	258 (-992,1509)	115
AUC (hr•ng/mL)	9524 (8319,10729)	12015 (9852,14177)	**7996[a] (6833,9149)**	**8375[b] (7219,9531)**	6200 (-23812,36212)	2753
HB/BUP	14.4 (12.6,16.2)	17.4 (14.1,20.8)	**12.5[c] (10.6, 14.4)**	**12.8[c] (10.8,14.8)**	12.2 (-34.2,58.6)	6.1
EB						
C_{max} (ng/mL)	38 (35,42)	43 (37,49)	35 (31,41)	36 (31,41)	38 (-1,77)	22
Css (ng/mL)	32 (29,36)	36 (30,42)	30 (25,34)	30 (26,34)	31 (-183,246)	19
AUC (hr·ng/mL)	772 (689,856)	868 (727,1008)	713 (609,817)	721 (618,825)	753 (-4393,5899)	444
EB/BUP	1.15 (1.05,1.25)	1.24 (1.07,1.42)	1.09 (0.96,1.22)	1.08 (0.93,1.23)	1.30 (-0.48,3.08)	0.99
TB						
C_{max} (ng/mL)	208 (181,236)	241 (190,293)	188 (157, 218)	188 (159,216)	222 (-9,453)	122
Css (ng/mL)	175 (150,200)	204 (153,255)	158 (132,182)	157 (132,182)	190 (-1220,1599)	108
AUC (hr•ng/mL)	4209 (3612,4807)	4893 (3661,6126)	3789 (3170, 4407)	3775 (3178,4371)	4549 (-29275,38373)	2582
TB/BUP	6.21 (5.53,6.90)	6.93 (5.53,8.32)	5.77 (5.04, 6.50)	5.62 (4.82,6.42)	7.50 (3.78,11.23)	5.73

Significant differences compared to no reduced function allele group

[a] $P = 0.005$

[b] $P < 0.001$

[c] $P = 0.01$

Table 3

24-hour Urine Bupropion and Metabolite Excretion

	24-hour recovery (nmol, mean = 95%)					
	All (*N*=42)	**No reduced function allele (*N*=16)**	**With reduced function allele(s) (N=26)**	***6 allele (*N*= 23)**	***18 allele (*N*= 2)**	***6/18 allele (*N*= 1)**
BUP	2087 (1512,2662)	2568 (1169,3968)	1790 (1346,2234)	1800 (1305,2295)	1301 (-3755,6356)	2543
HB – free	3672 (2782,4561)	4698 (2776,6619)	3041 (2187,3895)	3207 (2257,4157)	1736 (884,2587)	1824
HB – gluc	12283 (10393,14173)	14902 (11602,18201)	**12283 (8450, 12892)**	**11068 (8612,13525)**	9039 (-4153,22232)	4787
EB–free	4872 (3753,5991)	6186 (3634,8738)	4063 (3107,5020)	4067 (2996,5138)	3711 (-15768,23191)	4692
EB – gluc	1718 (1438,1998)	1905 (1354,2456)	1603 (1278,1928)	1602 (1235,1969)	1763 (-2118,5643)	1304
TB–free	38376 (27180,49572)	53034 (25558,80501)	29356 (22366,36344)	28990 (21286,36694)	32492 (-163420,228404)	31495
TB – gluc	4778 (2539,7017)	7731 (2047,13415)	2961 (1862,4060)	2877 (1788,3966)	5402 (-39287,50083)	0
	% Daily Bupropion dose					
	All (*N*=42)	**No reduced function allele** (*N*=16)	**With reduced function allele(s)** (N=26)	***6 allele** (*N*= 23)	***18 allele** (*N*= 2)	***6/18 allele** (*N*= 1)
BUP	0.33% (0.24%,0.43%)	0.41% (0.19%,0.63%)	0.29% (0.22%,0.36%)	0.29% (0.21%,0.37%)	0.21% (-0.60%,1.02%)	0.41%
HB – free	0.59% (0.45%,0.73%)	0.75% (0.44%,1.06%)	0.49% (0.35%,0.62%)	0.51% (0.36%,0.67%)	0.28% (0.14%,0.41%)	0.29%
HB – gluc	1.97% (1.66%,2.27%)	2.38% (1.86%,2.91%)	**1.70% (1.35%,2.06%)**	**1.77% (1.38%,2.16%)**	1.45% (-0.66%,3.56%)	0.77%
EB–free	0.78% (0.60%,0.96%)	0.99% (0.58%,1.40%)	0.65% (0.50%,0.80%)	0.65% (0.48%,0.82%)	0.59% (-2.52%,3.71%)	0.75%
EB – gluc	0.27% (0.23%,0.32%)	0.30% (0.22%,0.39%)	0.26% (0.20%,0.31%)	0.26% (0.20%,0.32%)	0.28% (-0.34%,0.90%)	0.21%
TB–free	6.14% (4.35%,7.93%)	8.49% (4.09%,12.88%)	4.70% (3.58%,5.82%)	4.64% (3.41%,5.87%)	5.20% (-26.18%,36.54%)	5.04%
TB – gluc	0.76% (0.41%,1.12%)	1.24% (0.33%,2.15%)	0.47% (0.30%,0.65%)	0.46% (0.29%,0.64%)	0.86% (6.29%,8.01%)	0.00%
	Renal clearance (ml/min/kg)					
	All (*N*=42)	No reduced function allele (*N*=16)	With reduced function allele(s) (N=23)	*6 allele (*N*= 23)	*18 allele (*N*= 2)	*6/18 allele (*N*= 1)
BUP	0.17 (0.12,0.21)	0.18 (0.10,0.26)	0.16 (0.10,0.21)	0.16 (0.09,0.22)	0.10 (-0.33,0.53)	0.28
HB	0.02 (0.02,0.03)	0.03 (0.02,0.04)	0.03 (0.02,0.03)	0.03 (0.02,0.03)	0.01 (-0.04,0.06)	0.04
EB	0.36 (0.28,0.45)	0.41 (0.26,0.56)	0.34 (0.24,0.43)	0.34 (0.23,0.45)	0.20 (-0.12,0.52)	0.53
TB	0.50 (0.39,0.61)	0.58 (0.36,0.81)	0.44 (0.33,0.56)	0.45 (0.32,0.58)	0.29 (-0.14,0.73)	0.62

Bold – significant difference compared to no reduced function allele group, p = 0.03.

Table 4

Regression Analysis of Predictors

Hydroxybupropion AUC‡ R^2 = 0.50 (p < 0.001)				
Predictor	**B**	**β**	**95% CI**	**P**
*CYP2B6*6* (per # alleles)	**-3049**	**-0.56**	**-4411, 1-687**	**0.001**
*CYP2B6*18* (per # alleles)	**-5095**	**-0.34**	**-9018, -1171**	**0.012**
Sex (female)	**2287**	**0.37**	**917, 4856**	**0.005**
Race (Asian)	-2071	-0.2	-4734, 592	0.123
Race (African American)	-1667	-0.21	-3893, 559	0.138
Hydroxybupropion/Bupropion R^2 = 0.32 (p = 0.01)				
Predictor	**B**	**β**	**95% CI**	**P**
*CYP2B6*6* (per # alleles)	**-4.22**	**-0.51**	**-6.60, -1.83**	**0.001**
*CYP2B6*18* (per # alleles)	-6.69	-0.30	-13.56, 0.18	0.056
Sex (female)	1.88	0.16	-1.57, 5.33	0.277
Race (Asian)	-4.00	-0.26	-8.66, 0.66	0.090
Race (African American)	-0.25	-0.02	-4.15, 3.65	0.897

‡AUC in hr*ng/mL

The B and β provided refers to the variables listed in parentheses beside each categorical predictor.