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Nicotine Dependence Pharmacogenetics: Role of Genetic Variation in Nicotine-Metabolizing Enzymes

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Abstract

Nicotine-dependence pharmacogenetics research is an emerging field, and a number of studies have begun to characterize the clinical relevance and predictive power of genetic variation in drug-metabolizing enzymes and drug target genes for response to medication. The present paper focuses on evidence for the role of nicotine-metabolizing enzymes in smoking behavior and response to treatment. Nicotine metabolism is mediated primarily by cytochrome P450 2A6 (CYP2A6). Genetic variation in the *CYP2A6* gene has been linked with several smoking behavior phenotypes. Individuals who carry null or reduced activity alleles for *CYP2A6* smoke fewer cigarettes per day, are less dependent on nicotine, and may have an easier time quitting smoking. A phenotypic measure of CYP2A6 enzyme activity, defined as the ratio of the nicotine metabolites 3′hydroxycotinine/cotinine, also predicts successful quitting with the transdermal nicotine patch, and counseling alone. Faster metabolizers of nicotine respond more poorly to these treatments; however, they may be excellent candidates for non-nicotine therapies, such as bupropion. Inherited variation in the CYP2B6 enzyme is also associated with response to bupropion treatment and counseling alone for smoking cessation. Inhibition of the CYP2A6 enzyme to slow nicotine metabolism is a promising approach to increase nicotine availability and potentially reduce harm from tobacco smoking.

Keywords

tobacco; nicotine; addiction; genetics; pharmacogenetics

With about 1 billion smokers worldwide, cigarette smoking is among the most significant public health problems (WHO, 2008). Mortality due to smoking is greater than that attributable to HIV, illegal drug and alcohol use, motor vehicle accidents, and murders combined (CDC, 2004). In the United States, 21% of adults are current smokers, and this rate has remained stable for the past few years (MMWR, 2007). Rates of tobacco use in developing nations are rising rapidly, and it is predicted that as many as 500 million people across the world will suffer from tobacco-related mortality (Levine & Kendler, 2004; WHO, 2008). Cigarette smoking causes 80–90% of all lung cancer deaths and also increases the risk of other cancers (e.g., bladder, oral cavity, and esophagus), cardiovascular disease (e.g.,

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myocardial infarction, stroke), lung disease (e.g., emphysema, bronchitis), and risk of infectious diseases (CDC, 2004). The economic burden of such widespread illness due to smoking is roughly hundreds of billions of dollars every year (Guindon, 2006). Therefore, effective treatments for smoking cessation are an important public health priority.

Currently, approved therapies for smoking cessation include nicotine replacement therapy (NRT; transdermal patch, nasal spray, gum, inhaler, lozenge), buproprion (Wellbutrin® or Zyban®), and varenicline (Chantix®) (Schnoll and Lerman, 2006). NRTs are associated with 1.5–2-fold increased abstinence rates relative to placebo (Stead et al., 2008). The antidepressant, bupropion, also doubles quit rates, compared to placebo (Hughes et al., 2007; Jorenby et al., 1999). Although its mechanism of action is not completely understood, bupropion is a weak inhibitor of dopamine and norepinephrine reuptake in the brain (Sanchez & Hyttel, 1999). Varenicline, a α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist, is the most recent medication to be approved by the U.S. Food and Drug Administration for the treatment of nicotine dependence. As a partial agonist at the α4β2 nAChRs, varenicline is designed to decrease craving and withdrawal symptoms, in addition to reducing the rewarding value of a cigarette during a lapse (Coe et al., 2005; Rollema et al., 2007). Based on meta-analysis, the pooled cessation odds ratio for varenicline relative to placebo is 3.22, and greater efficacy compared to bupropion and NRT has been demonstrated (Aubin et al., 2008; Cahill et al., 2007; Gonzales et al., 2006). Several other medications are considered second-line treatments for nicotine dependence, including nortriptyline and clonidine (Gourlay et al., 2004; Hughes et al., 2007; Schnoll and Lerman, 2006).

The science and treatment of nicotine addiction is being advanced by emerging research in pharmacogenetics. Studies have begun to characterize the role of genetic variation in drug-metabolizing enzymes and drug-target genes for response to different types of medications for smoking cessation. The reader is referred to recent reviews of this field for a broader view of pharmacogenetics and nicotine-dependence treatment (Lee & Tyndale, 2006; Lerman et al., 2007). Here, we focus on the role of inherited variation in nicotine-metabolizing enzymes in smoking behavior, smoking cessation, and response to therapy. This review begins with an overview of nicotine metabolism, including the role of specific genetic variants. The sections that follow discuss associations of genetic variation in nicotine-metabolizing enzymes with smoking behavior and response to medication. The final sections discuss the role of these genetic variants in tobacco harm, implications of genetics research for medication development, and issues in the translation of genetics research to clinical practice.

NICOTINE METABOLISM

Nicotine inhaled via smoking is rapidly absorbed from the lung into the systemic circulation in a matter of seconds, bypassing the first-pass metabolism in the liver (Benowitz, 1990). Approximately 80–90% of nicotine is converted to its inactive form cotinine, primarily by the cytochrome P450 enzyme, CYP2A6 (Benowitz & Jacob, 1994; Nakajima et al., 1996). Cotinine is then metabolized to *trans*-3′hydroxycotinine (3-HC) by CYP2A6, and 3-HC is the most abundant nicotine metabolite in the urine (Benowitz, 2008). With regular smoking, plasma cotinine levels are about 15-fold greater than nicotine levels and plasma *trans*-3′hydroxycotinine levels are about 3-fold greater than nicotine levels (Benowitz, 1998). The CYP2A6 enzyme is also responsible for the activation of procarcinogenic tobacco-specific nitrosamines (Patten et al., 1997; Yamazaki et al., 1992), as is discussed in greater detail in below.

The CYP2A6 enzyme accounts for the vast majority of the metabolism of nicotine to cotinine, with minor contributions from CYP2B6, CYP2D6, and CYP2E1 enzymes (Messina et al., 1997; Nakajima et al., 1996; Yamanaka et al., 2005; Yamazaki et al., 1999). CYP2B6 has an approximately 10% catalytic efficiency of the CYP2A6 enzyme *in vitro* in nicotine c-oxidation, and it might play a minor role in nicotine clearance at higher nicotine levels or in *CYP2A6* genetically impaired individuals (Benowitz et al., 2006b; Yamazaki et al., 1999). While CYP2A6 is expressed primarily in the liver, CYP2B6 is expressed at higher levels in the brain, where it may influence highly localized metabolism of nicotine in the brains of human smokers (Miksys et al., 2003).

Twin studies provide consistent support for the heritability of nicotine metabolism (Swan et al., 2004, 2005). Accordingly, several functional polymorphisms in the CYP2A6 gene that affect enzyme activity have been characterized (Fernandez-Salguero et al., 1995; Goodz and Tyndale, 2002; Mwenifumbo et al., 2008a) (http://www.imm.ki.se/CYPalleles/cyp2a6.html). To date, the most widely studied polymorphisms are the *CYP2A6**2 (L160H amino-acid substitution, lacking activity) (Yamano et al., 1990), *CYP2A6**4 (deletion variant lacking activity) (Kitagawa et al., 1999; Nunoya et al., 1998), *CYP2A6**9 (48T>G substitution in the TATA promoter region; 50% reduced enzyme activity) (Nakajima et al., 2006), and *CYP2A6**12 (10 amino-acid substitution; decreased enzyme activity) (Benowitz et al., 2006b). The *2 and *4 variants are more common in Asian populations than in persons of European ancestry (Malaiyandi et al., 2005; Nakajima et al., 2001). Since all of these reduced activity alleles have relatively low frequency in the general population, genetic subgroups of individuals have been combined in some studies to represent those with variants associated with slow (less that 50% CYP2A6 activity), intermediate (80% CYP2A6 activity), and normal metabolism (100% CYP2A6 activity; Benowitz et al., 2006b). Included among the slow metabolizers are those who have one or two copies of the null (no activity) alleles (*CYP2A6**2 or *CYP2A6**4), or have two copies of the reduced activity alleles (*CYP2A6**9 or *CYP2A6**12). Intermediate metabolizers include carriers of a single *CYP2A6**9 or *CYP2A6**12 allele, and normal metabolizers are those with *1/*1 (wild-type genotypes). The population frequencies of the reduced activity alleles are 7–9% in Caucasians, 8% in African Americans, 16% in Chinese, 22% in Korean, and 21% in Japanese populations (Malaiyandi et al., 2005; Schoedel et al., 2004). The population frequencies of null alleles are 1.2% in Caucasians, 2% in African Americans, 7–15% in Chinese, 11% in Korean, and 20–24% in Japanese (Nakajima et al., 2006; Rao et al., 2000; Schoedel et al., 2004). The *CYP2A6**1×2 allele is a duplication variant that is associated with higher rates of nicotine metabolism than the wild-type CYP2A6 (Rao et al., 2000). The *CYP2A6**1B variant is associated with approximately a 20% higher clearance of nicotine and 30% greater nicotine-metabolite ratio (Mwenifumbo et al., 2008b). It should be noted that many new *CYP2A6* alleles are being identified and characterized in different populations, indicating that the proportion of those with slower and faster nicotine metabolism, detected by genetic testing, is increasing over time (Ho et al., 2008; Mwenifumbo et al., 2008a). A listing of *CYP2A6* alleles and corresponding activity is provided in Table 1.

Several functional variants in the *CYP2B6* gene have also been identified. These include *CYP2B6**4 (A785G; increased activity) (Lang et al., 2001), *CYP2B6**9 (G516T; increased activity) (Ariyoshi et al., 2001), *CYB2B6**5 (C1459T; decreased protein and enzyme activity) (Lang et al., 2001; Miksys et al., 2003), and *CYP2B6**6 (G516T and A785G; structurally altered enzyme with little impact on peripheral nicotine metabolism) (Lee et al., 2007b) (http://www.cypalleles.ki.se/cyp2b6.htm). There is a distinct role of the CYP2B6 enzyme in the metabolism of the smoking-cessation medication, bupropion (Faucette et al., 2000), and the prevalent *CYP2B6**6 variant is associated with slower bupropion metabolism

(Hesse et al., 2004); however, it may increase the metabolism of other substrates (Jinno et al., 2003; Xie et al., 2003).

Finally, individual variation in nicotine metabolism may also be assessed by using a phenotypic measure, namely the ratio of the nicotine metabolites derived from cigarette smoking (3-HC/cotinine) (Benowitz et al., 2003; Dempsey et al., 2004). The 3-HC/cotinine ratio can be measured reliably in saliva or plasma (Dempsey et al., 2004), has minimal diurnal variation (Lea et al., 2006), and is independent of smoking patterns or time since last cigarette (Levi et al., 2007), at least among relatively regular smokers. Null or reduced activity *CYP2A6* alleles (*2, *4, *9, and *12) are associated with lower 3-HC/cotinine ratios and slower metabolism (Dempsey et al., 2004; Johnstone et al., 2006; Malaiyandi et al., 2006a). The nicotine metabolite ratio may be optimal for assessing individual differences in nicotine-metabolism rate, because it accounts for environmental factors such as ethnicity, sex, and age, which may alter the nicotine metabolic rate (Benowitz et al., 2006a; Johnstone et al., 2006; Mwenifumbo and Tyndale, 2007).

ASSOCIATIONS OF GENETIC VARIATION IN NICOTINE-METABOLIZING ENZYMES AND SMOKING BEHAVIOR

Nicotine is the addictive chemical in cigarette smoke, and the rate at which a smoker inactivates nicotine, based on *CYP2A6* genotype, influences a variety of smoking-behavior phenotypes. These include smoking adoption, smoking status and rate/level, dependence, and cessation. It is conjectured that individuals who carry the genetic predisposition to be faster metabolizers of nicotine would be more prone to develop a dependent smoking habit and would smoke more to maintain nicotine levels in a desired range (i.e., to offset the faster conversion of nicotine to its inactive form, cotinine) (Audrain-McGovern et al., 2007). Further, faster metabolizers may have greater difficulty quitting smoking, due perhaps to increased abstinence symptoms. As described below, each of these hypotheses have received partial support.

With regard to smoking adoption, two prospective studies have been conducted to date. However, the results of these studies are not consistent with respect to the risk of developing tobacco dependence in slower metabolizers. O'Loughlin and colleagues followed over 1,200 7th-grade students every 3–4 months for 4 years to study predictors of progression of smoking adoption (Karp et al., 2006; O'Loughlin et al., 2004). The incidence of conversion to tobacco dependence (had =3 of the criteria from the International Statistical Classification of Diseases [ICD] Version 10) was three times greater among the slower metabolizers (*CYP2A6**2 or *CYP2A6**4) (Karp et al., 2006), who also had a trend toward lower cigarette consumption, as compared to normal metabolizers (O'Loughlin et al., 2004). A recent study of smoking adoption among 222 adolescents followed from 9th to 12th grade reported that those with normal rates of metabolism (*CYP2A6 *1/*1*) progressed in degree of nicotine dependence (increase in Fagerstrom Test for Nicotine Dependence >1) more quickly than slow metabolizers (*CYP2A6**9, *CYP2A6**12, *CYP2A6**2, or *CYP2A6**4) (Audrain-McGovern et al., 2007). As seen in other adolescent and adult smokers, cigarette consumption was lower among slow than normal metabolizers (Audrain-McGovern et al., 2007). Differences in ages, heaviness of smoking, or methods of assessment of nicotine dependence may have contributed to these differing results on smoking acquisition in youth. Alternatively, it is possible that early in smoking, slow metabolizers may convert to dependence more rapidly, but the rate of increase in level of dependence and rate of smoking may increase more rapidly for the normal metabolizers relative to slow metabolizers. Additional research is necessary to sort out the role of the *CYP2A6* gene in smoking adoption.

Associations between *CYP2A6* genotype and smoking status in adults have been evaluated in several case-control studies (Munafo et al., 2004). Specifically, reduced or null activity *CYP2A6* alleles are significantly more prevalent among nonsmokers, as compared to smokers of Caucasian (Malaiyandi et al., 2005; Pianezza et al., 1998), Japanese (Iwahashi et al., 2004), and African-American descent (Mwenifumbo et al., 2007). Among individuals who smoke, those with reduced activity or null variants of *CYP2A6* tend to be lighter smokers (Fujieda et al., 2004; Malaiyandi et al., 2006b; Malaiyandi et al., 2005; Minematsu et al., 2006; Mwenifumbo et al., 2007; Rao et al., 2000; Schoedel et al., 2004; Tyndale et al., 1999) and are also less dependent on nicotine (Kubota et al., 2006; Malaiyandi et al., 2006b). Further, decreased cigarette consumption in slower metabolizers is associated with lower levels of plasma cotinine and breath carbon monoxide (CO) readings (Rao et al., 2000). These differences may be due to smoking fewer cigarettes, as well as to the decreased puff volume observed during smoking in slow metabolizers, compared to smokers who carry wild-type alleles (Strasser et al., 2007).

Fewer studies have examined associations of *CYP2A6* with smoking cessation. Retrospective studies have demonstrated that individuals with the low activity alleles of *CYP2A6* are twice as likely to quit smoking (Gu et al., 2000) and are less likely to experience severe withdrawal after a quit attempt (Kubota et al., 2006), compared to smokers with the wild-type genotype. As discussed in more detail below, further studies have begun to explore the relationship of inherited variation in nicotine-metabolizing enzymes in clinical trials of pharmacotherapies for nicotine dependence.

ASSOCIATIONS OF GENETIC VARIATION IN NICOTINE- METABOLIZING ENZYMES AND RESPONSE TO TREATMENT

A recent pharmacogenetic trial of NRT with over 300 participants examined whether *CYP2A6* genotype (or phenotype based on 3-HC/cotinine ratio) predicts treatment outcome (Malaiyandi et al., 2006b). Among participants in this open-label trial of nicotine patch versus nicotine nasal spray (Malaiyandi et al., 2006b), slower metabolizers (i.e., those with one null allele or two reduced activity alleles) had significantly higher treatment levels of plasma nicotine from the patch than normal metabolizers, with equivalent rates of compliance. In contrast, among those randomized to receive nicotine nasal spray, the slow metabolizers self-administered fewer doses of the spray, as compared to the normal metabolizers, resulting in equivalent levels of plasma nicotine (Malaiyandi et al., 2006b). A second study reported that smokers with the reduced activity *CYP2A6* variants were more sensitive to the effects of the patch, causing them to relapse after a quit attempt (Ozaki et al., 2006); however, the sample size ($n = 41$) was too small to be conclusive.

The small number of smokers with reduced or null activity alleles in the Malaiyandi et al. (2006b) trial complicated the analysis of smoking cessation rates; therefore, this was examined by using the phenotypic marker of *CYP2A6* activity (3-HC/cotinine ratio) (Lerman et al., 2006). As noted above, higher scores for the 3-HC/cotinine ratio reflect increased *CYP2A6* activity (i.e., faster metabolism), while lower scores reflect decreased *CYP2A6* activity (i.e., slower metabolism). Among individuals in the nicotine-patch condition ($n = 193$), there was a significant dose-response effect of nicotine metabolism rate (characterized by quartiles) on quitting at the end of 8 weeks treatment (from 46 to 27%) and at 6-month follow-up (from 30 to 11%). In fact, there was a 30% drop in the odds of remaining abstinent with each increasing quartile of the metabolite ratio (i.e., as nicotine metabolism increased, abstinence rate decreased) (Lerman et al., 2006). No such relationship was observed among smokers who received nicotine nasal spray ($n = 201$), presumably because they titrated their dose of treatment based on phenotype/genotype (Malaiyandi et

al., 2006b). *CYP2B6* genotype was unrelated to abstinence success in either the nicotine-patch or the nicotine-spray conditions in this trial (Lee et al., 2007b).

There are a few plausible mechanisms that may underlie associations of individual differences in nicotine metabolism with smoking cessation in the nicotine-patch condition (Lerman et al., 2006). There is some evidence that slower metabolizers may have lower levels of abstinence-induced cravings. Indeed, among the participants who had successfully stopped smoking after 1 week of nicotine-patch therapy, the metabolite ratio was significantly associated with the intensity of self-reported cravings, and these, in turn, predicted abstinence (Lerman et al., 2006). However, the most obvious explanation is that slower metabolizers obtain higher treatment levels of nicotine from the patch than higher metabolizers. Indeed, this was found in the trial (Lerman et al., 2006); however, treatment levels of plasma nicotine accounted for a very small proportion of the variance in quitting. Further, the presence of an association of nicotine metabolism with abstinence at 6-month follow-up, after treatment was discontinued, argues against differences in metabolism of nicotine from the patch being the only reason for the improved cessation rates. Thus, nicotine metabolism rate may influence quitting success independent of treatment, and this hypothesis was addressed in the placebo-controlled trial discussed below.

The role of *CYP2A6* activity, based on the pretreatment nicotine-metabolite ratio (3-HC/cotinine), has also been examined in this bupropion pharmacogenetic cessation trial (Patterson et al., 2008). In the placebo condition (i.e., counseling alone), there was a dose-response effect of nicotine metabolism; end-of-treatment quit rates across the quartiles were (from slowest to fastest metabolism): 32, 25, 20, and 10%. This further substantiates the role of variation in CYP2A6-mediated nicotine metabolism in altering smoking behaviors, in this case quitting smoking. Bupropion improved quit rates among fastest metabolizers (4th quartile) from 10 to 34% at the end of treatment (Patterson et al., 2008). The slowest metabolizers had equivalent quit rates of 32% on bupropion and placebo. These data, together with the NRT study described above (Lerman et al., 2006), suggest that CYP2A6 slow metabolizers might benefit from a nicotine patch or counseling alone, while faster metabolizers may be excellent candidates for bupropion or another non-nicotine medication.

As described earlier, there are multiple *CYP2B6* variants that are reported to have functional effects, and two of these have been examined for associations with smoking cessation in bupropion pharmacogenetic trials. Although *CYP2B6* appears to have little influence in peripheral nicotine metabolism, expression of this enzyme in the brain suggest potential influences on nicotine-addiction phenotypes, such as ability to quit smoking (Miksys et al., 2003). Of particular relevance to bupropion, the CYP2B6 enzyme is the primary enzyme involved in the metabolism of bupropion to its metabolites, hydroxybupropion, erthrohydrobupropion, and threohydrobupropion (Faucette et al., 2000); however, the parent compound and metabolites are biologically active, and thus, rates of bupropion metabolism may not affect outcome. The *CYP2B6**5 (C1459T) variant has been associated with reduced protein expression and bupropion metabolism (Hesse et al., 2004; Lang et al., 2001). The *CYP2B6**6 (G516T and A785G) variant has also been associated with decreased bupropion metabolism (Hesse et al., 2004; Loboz et al., 2006). These variants in the *CYP2B6* gene are in high linkage disequilibrium (Johnstone et al., 2006), and many studies have focused on individual SNPs rather than alleles (e.g., C1459T is also found in *7).

The *CYP2B6* C1459T SNP was examined among 426 participants of European ancestry in a pharmacogenetic placebo-controlled trial of bupropion for smoking cessation. Compared to smokers with the wild-type genotype, those with one or two T variants who received placebo reported higher levels of abstinence-induced cravings and were less likely to be abstinent at the end of treatment (Lerman et al., 2002). Bupropion treatment reversed the

increased relapse risk among female carriers of the T variant. Indeed, among females with the variant, end-of-treatment abstinence rates were 15% on placebo versus 54% on bupropion (Lerman et al., 2002). In an independent bupropion placebo-controlled trial, the T variant at 1459 (one or two copies) moderated the effect of the *ANKK1* Taq1A polymorphism on abstinence (David et al., 2007).

A more recent analysis from the first bupropion trial examined the role of the *CYP2B6**6 variant on treatment response. Smokers with the *CYP2B6**6 genotype (one or two copies of *CYP2B6**6) performed poorly on placebo, but had a positive treatment response with bupropion (end-of-treatment quit rates of 14.3% on placebo vs. 32.5% on bupropion for this group), that was well-maintained at 6-month follow-up (12.9% on placebo vs. 31.2% on bupropion for this group). Those possessing the wild-type genotype had high quit rates regardless of treatment (31.6% on placebo vs. 31.0% on bupropion) (Lee et al., 2007a). Since the effect of genotype in this study was observed mainly in the placebo group, the *CYP2B6**1 versus the *CYP2B6**6 allele may exert their differing effects on relapse risk by altering brain metabolism of nicotine. This hypothesis is being explored in further investigations.

NICOTINE METABOLIZING ENZYMES AND TOBACCO HARM

It is well known that cigarette smoking is a prominent cause of cancers of the lung, head and neck, and other vital organs (Peto et al., 2000). In addition to its influence on nicotine metabolic inactivation, CYP2A6 also activates tobacco-specific procarcinogens, including nitrosamines, such as NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), NDEA (N-nitrosodiethylamine), and NNN (N'-nitrosonornicotine), due to their structural similarities to nicotine. Carcinogen formation from precursor compounds is reduced in persons with reduced or null activity *CYP2A6* alleles and also with CYP2A6 inhibition (Sellers et al., 2003a). Case-control studies have demonstrated that the null-activity *CYP2A6**4/*4 genotype is less prevalent among lung cancer cases than healthy smoker controls, even after controlling for the impact on reducing smoking levels (Ariyoshi et al., 2002; Fujieda et al., 2004; Kamataki et al., 1999; Miyamoto et al., 1999). Thus, persons with higher levels of *CYP2A6* activity (i.e., faster metabolizers of nicotine) not only are at risk for tobacco dependence and smoking persistence, but also are at increased risk for tobacco-related cancer if they smoke.

IMPLICATIONS FOR MEDICATION DEVELOPMENT

The protective effects of null or reduced activity *CYP2A6* alleles on both nicotine dependence and lung cancer suggests that inhibition of CYP2A6 may be a useful therapeutic strategy (Sellers et al., 2003b). Methoxsalen, a medication that is approved for the treatment of psoriasis, is a potent inhibitor of CYP2A6 (Damaj et al., 2007; Sellers & Tyndale, 2000; Zhang et al., 2001). In pharmacokinetic assessments, methoxsalen, at doses of 10 and 30 mg administered to overnight abstinent smokers, doubled plasma nicotine levels from 4 mg of oral nicotine and decreased the self-rated desire to smoke (Sellers et al., 2000). When smokers were allowed to smoke *ad libitum* after the administration of 30 mg of methoxsalen along with 4 mg of oral nicotine, there was a decrease in expired breath CO by 47%, cigarettes smoked by 24%, total numbers of puffs taken, and an increase in the latency to light a cigarette, compared to smokers who received placebo plus 4 mg of oral nicotine (Sellers et al., 2000). Methoxsalen (10 mg) administered to smokers who were asked to maintain their smoking habit for 3 days caused a significant rerouting of NNK metabolism to the inactive NNAL-glucuronide, presumably by decreasing the metabolic activation of the procarcinogen NNK (Sellers et al., 2003a). Thus far, no clinical trials have been conducted for methoxalen or other CYP2A6 inhibitors as a smoking-cessation medication. Selegiline, a

monoamine oxidase B (MAO-B) inhibitor, has been tested in some small studies as a potential smoking cessation medication (Biberman et al., 2003; George et al., 2003). Preclinical studies have also demonstrated that selegiline inhibits human CYP2A6 and mouse CYP2A5 enzyme activity *in vitro* and decreases mouse nicotine clearance *in vivo* by approximately 40%, supporting its potential as a nicotine-dependence medication (Siu & Tyndale, 2008). Newer *in-silico* approaches are now being explored for CYP2A6 inhibition and its therapeutic applications (Rahnasto et al., 2007).

TRANSLATION OF GENETICS RESEARCH TO PRACTICE

There are a number of stages of research that are necessary prior to the translation of pharmacogenetics research to practice, in the form of genetically tailored treatment (Shields et al., 2004). First, and most important, is the independent replication of findings across multiple studies. As pharmacogenetics and nicotine dependence is an emerging science, this criterion has yet to be achieved in most cases; however, evidence for associations of *CYP2A6* with smoking behavior and for the nicotine-metabolite ratio as a predictor of relapse appear very promising. In addition to the demonstration of efficacy in independent settings, it is important to evaluate whether pharmacogenetic tailoring is more cost effective than simply providing medication to all smokers in treatment (Grossman, 2007; Shields et al., 2004). Cost effectiveness of implementing tailored therapy would depend on the distribution of the relevant genetic polymorphisms, costs involved in genotyping individuals, and subsequent effectiveness of the tailored versus untailored therapy (Roden et al., 2006). Our group recently completed a study in which cost effectiveness of smoking cessation tailored, based on genotype, was estimated and compared to the standard modalities of treatment available by using simulations run on the pharamacogenetic data available from our bupropion and NRT pharmacogenetic trials described above (Heitjan et al., 2008). Genetically tailored therapy was found to be more effective and less costly than standard NRT treatment; however, it was less efficacious and cost effective than bupropion or varenicline therapy (Heitjan et al., 2008). However, genetically tailored therapy may be more cost effective if the favorable genotype is neither too rare nor too common, if the interaction between treatment and genotype is substantial, and if the short-term outcome of therapy is a good predictor of longer term outcome (Heitjan et al., 2008). Further studies are necessary to examine the cost effectiveness of smoking-cessation therapy tailored based on the nicotine-metabolite ratio or genetic variation in nicotine-metabolizing enzymes.

Dissemination of information and enhancements in training of primary care physicians to deliver pharmacogenetically tailored therapy also represents a significant challenge for translation to practice (Shields & Lerman, 2008). Additional ethical and health policy issues to be addressed include the potential for discrimination and stigmatization, based on collateral information obtained through genetic testing (Shields et al., 2004). The potential for a clinical impact of research on genetic variation in nicotine-metabolizing enzymes will be greatest if research on these ethical and health policy issues is conducted in parallel with the clinical investigations. A transdisciplinary team approach to achieve these goals is likely to have the greatest public health impact.

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Table 1

CYP2A6 alleles with known *in vivo* impact on activity

Allele	Description of *in vivo* activity for nicotine
*1A	"Normal" activity (wild type)
*1B	Increased activity (faster nicotine clearance)
*2	Inactive
*4A-D	Inactive
*7	Inactive
*9A-B	Reduced activity
*10	Inactive
*12A-C	Reduced activity
*17	Inactive

This table includes the subset of *CYP2A6* alleles for which *in vivo* activity has been characterized. The reader is referred to Mwenifumbo and Tyndale (2007) for a more comprehensive listing and characterization of *CYP2A6* alleles and functional properties. Additional novel alleles identified in persons of Black African descent are reported elsewhere (Mwenifumbo et al., 2008a).