The Impact of *CYP2A6* Genotype on Smoking Cessation in an Extended Nicotine Patch Therapy Clinical Trial

By

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ABSTRACT

We investigated the efficacy of standard (8-week nicotine, 16-week placebo) vs extended (24-week nicotine) patch therapy for smoking cessation, and the effect of slow nicotine metabolism, indicated by *CYP2A6* reduced metabolizer (RM) genotype or low 3-hydroxycotinine/cotinine ratio (3HC/COT), on abstinence. RM versus normal genotype predicted lower 3HC/COT. Extended vs standard treatment produced higher abstinence at 24 weeks (32% vs 20%), but not at 52 weeks (both 14%). Low 3HC/COT and RM genotype predicted higher abstinence on extended versus standard treatment at 24 (47% vs 25%, 38% vs 17%) and 28 weeks (34% vs 19%, 23% vs 11%), while high 3HC/COT or normal genotype did not. Within extended treatment, low versus high 3HC/COT predicted higher abstinence at 8 (48% vs 29%), 24 (47% vs 25%), and 28 weeks (34% vs 16%), with similar trends for the genotype effect. Overall, extending nicotine treatment increased abstinence during therapy, particularly for slow metabolizers.

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Table of Contents

List of Tables

List of Figures

LIST OF ABBREVIATIONS

3HC/COT	3-Hydroxycotinine/Cotinine
3HC	3-Hydroxycotinine
COT	Cotinine
CPD	Cigarettes per day
CYP	Cytochrome P450
DSM-IV	Diagnostic and Statistical Manual of the American Psychiatric Association Version IV
EOT	End-of-treatment
FTND	Fagerström Test of Nicotine Dependence
IM	Intermediate metabolizer
nAChR	Nicotinic acetylcholine receptor
NM	Normal metabolizer
NRT	Nicotine replacement therapy
PCR	Polymerase chain reaction
RM	Reduced metabolizer
SM	Slow metabolizer
SNP	Single nucleotide polymorphism

1. INTRODUCTION

1.1 Smoking

There are over 1 billion smokers worldwide (WHO 2008). In Canada, there are 4.9 million smokers, which is 18% of the Canadian population aged 15 years and older (CTUMS 2008). The widespread use of tobacco has considerable consequences because it is the single most preventable cause of death in the world, resulting in over 5 million deaths per year worldwide (WHO 2008). It causes cancer, cardiovascular diseases, respiratory diseases, harms reproduction, immunity, and increases your risk of fractures, dental diseases, eye diseases and peptic ulcers (CDC 2004).

The aetiology of smoking is complex and includes environmental factors, such as the influence of peers and culture, as well as genetics. Twin, adoption, and family studies suggest that there is considerable genetic contribution to many aspects of smoking (Malaiyandi *et al.* 2005; Ho and Tyndale 2007; Lerman *et al.* 2007). For instance, 60-70% of the variability in nicotine dependence and smoking persistence is due to genetics (Carmelli *et al.* 1992; True *et al.* 1997; Heath *et al.* 1999; Koopmans *et al.* 1999; Kendler *et al.* 2000; McGue *et al.* 2000; Li *et al.* 2003; Broms *et al.* 2006). The number of cigarettes smoked and nicotine withdrawal symptoms were also found to have genetic components (45-86% and 26-48%, respectively) (Swan *et al.* 1990; Swan *et al.* 1996; Swan *et al.* 1997; Koopmans *et al.* 1999; Xian *et al.* 2003; Lessov *et al.* 2004; Vink *et al.* 2004; Broms *et al.* 2006). Other studies found that 51-54% of variance in the risk for failed smoking cessation was attributable to genetics (Xian *et al.* 2003; Xian *et al.* 2005).

1.2 Nicotine Dependence

1.2.1 Nicotine Titration

To date, over 4000 compounds have been identified in tobacco smoke, including toxins and carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines (Moritsugu 2007). Tobacco also contains several psychoactive ingredients, including nicotine and other minor alkaloids (e.g. nornicotine), acetaldehyde, and mono-amine oxidase inibitors (Dome *et al.* 2009). However, nicotine is the main psychoactive ingredient in tobacco, which is responsible for the pharmacological effects of tobacco, the maintenance of smoking behaviour and tobacco dependence (Henningfield *et al.* 1985; Surgeon's General Report 1988). Smokers titrate their smoking behaviour to maintain optimum nicotine levels in the brain and plasma (McMorrow and Foxx 1983). For instance, when the nicotine content of cigarettes is decreased, smoking behaviour is increased by methods such as smoking more cigarettes, increasing puff volume and puff frequency (Zacny and Stitzer 1988; Djordjevic *et al.* 1995; Scherer 1999; Kassel *et al.* 2007). Smoking behaviour also intensifies when renal nicotine clearance is increased by urine acidification (Benowitz and Jacob 1985). A decrease in plasma nicotine has also been shown to increase craving for cigarettes (Jarvik *et al.* 2000).

1.2.2 Reinforcing Effects of Nicotine

The establishment and maintenance of cigarette smoking is due, at least in part, to the reinforcing effects of nicotine on brain reward pathways (Corrigall and Coen 1989; Le Foll and Goldberg 2005; Le Foll and Goldberg 2006; Le Foll *et al.* 2007). Nicotine readily crosses the blood-brain barrier and activates α4β2-nicotinic acetylcholine receptors (nAChRs), located on the dopamine neurons in the ventral tegmental area (VTA) (Wonnacott 1997; Picciotto *et al.* 1998; Tapper *et al.* 2004; Lockman *et al.* 2005). This leads to increased dopamine release in the

nucleus accumbens and stimulation of the mesolimbic brain reward system (Pontieri *et al.* 1996; Pidoplichko *et al.* 1997; Stein *et al.* 1998; Laviolette and van der Kooy 2003; Rossi *et al.* 2005).

Chronic exposure to nicotine leads to the upregulation of α4β2-nAChRs in the central nervous system, and this response is believed to be due to nicotine-induced desensitization and long-term inactivation of nAChRs (Marks *et al.* 1983; Schwartz and Kellar 1985; Fenster *et al.* 1999; Quick and Lester 2002). Desensitization and inactivation of neuronal nAChRs is believed to play an important role in nicotine addiction by increasing tolerance and exacerbating withdrawal symptoms due to the upregulation of nAChRs (i.e. hyper-exciting cholinergic systems outside of the reward pathway) (Dani and Heinemann 1996). Tobacco withdrawal symptoms include anger, anxiety, craving, difficulty concentrating, hunger, impatience and restlessness (Hughes *et al.* 1991).

1.2.3 Dependence Measures

Tobacco or nicotine dependence can be diagnosed using standardized psychiatric diagnostic criteria, such as the *Diagnostic and Statistical Manual of Mental Disorders* fourth edition (DSM-IV) by the American Psychiatric Association and the *International Classification of Diseases* 10th revision (ICD-10) by the World Health Organization. Although administering DSM-IV or ICD-10 diagnostic interviews are considered ideal, they are difficult to implement in clinical or research settings because they are lengthy, expensive and require trained interviewers (Kawakami *et al.* 1999; Etter 2008). For these reasons, shorter, easy-to-apply, self-rating questionnaires based on the ICD-10 and DSM-IV definitions of dependence have been developed and are commonly used, such as the Fagerstrom Test of Nicotine Dependence (FTND). The six-item FTND is a revision of the Fragerstrom Tolerance Questionnaire (FTQ), and it is the most widely used and best-documented test of nicotine dependence in research or

clinical settings (Heatherton *et al.* 1991; Stavem *et al.* 2008). Other measures that are sometimes used include the Heaviness of Smoking Index, which is a shorter version of FTND containing two of the six items, or the twelve-item Cigarette Dependence Scale (Etter 2008).

1.3 Smoking Cessation

Of the 70% of smokers that say they want to quit, only 40% make a quit attempt each year (CDC 2002; CDC 2005). In instances of unaided quit attempts, the majority of smokers relapse within the first week of quitting, resulting in abstinence rates at 6 months of approximately 3-5% (Hughes *et al.* 2004). However, pharmacotherapy for smoking cessation can increase the odds of attaining long-term abstinence by 2-3 fold, compared to placebo (Nides 2008). Currently, the first-line therapeutic agents for smoking cessation approved by the US Food and Drug Administration (FDA) and Health Canada include multiple formulations of nicotine replacement therapy (NRT), Bupropion (Zyban) and Varenicline (Chantix) (Le Foll and George 2007; Nides 2008). The pharmacological and clinical properties of these therapies are summarized in Table 1.1.

1.3.1 NRT

NRT is the recommended first-line treatment for nicotine dependence. The goal of NRT is to reduce the desire to smoke by partially replacing the nicotine obtained from cigarettes in order to alleviate nicotine withdrawal symptoms and cravings, and to reduce the reinforcing and cognitive effects of nicotine (Nides 2008; Stead *et al.* 2008). There are six NRT formulations (transdermal patch, nasal spray, gum, lozenge, inhaler and sublingual tablet) that differ in route of administration, dose, duration and minor side effects (summarized in Table 1.1). The suggested duration of treatment is 8-12 weeks, however it can be used for longer to prevent relapse. While

Table 1.1: First-line pharmacotherapies for smoking cessation.

Therapy	Mechanism	Comments	Dose	Duration	Efficacy	Contra-indications	Adverse effects
Nicotine replacement therapy (NRT)	partially replaces nicotine from cigarettes: alleviates withdrawal and cravings, with reduced reinforcing and cognitive effects	Patch: does not replace behavioural activities of smoking or provide much positive reinforcement Gum, Lozenge: self-titration Inhaler: self-titration; somewhat mimicks behavioural aspects of smoking Spray: self-titration; most rapid-delivery formulation; provides most positive reinforcement and fastest relief of withdrawal	Patch: 5/10/15 mg nicotine over 16 hrs or 7/14/21 mg nicotine over 24 hrs Gum: 2/4 mg Inhaler: 4 mg/puff Spray: 1 or 2 0.5 mg/ nostril Lozenge: 2/4 mg	8-12 wks, or longer to prevent relapse	2x odds of quitting vs placebo	pregnancy; use with caution in acute cardiovascular conditions (e.g. recent AMI)	FORMULATION SPECIFIC Patch: mild skin irritation at patch site Gum: jaw pain, mouth soreness, dyspepsia, hiccoughs Inhaler: mouth and throat irritation, cough Spray: runny nose, throat and nasal irritation, cough Lozenge: mouth and throat irritation, hiccoughs
Bupropion	inhibits reuptake of dopamine and norepinephrine; weak antagonism of nAchR	decreases craving and withdrawal; monotherapy or in combination with NRT; equally effective in those with/without history of depression	150 mg SR q.d PO for first 3 days, then 150 mg SR b.i.d PO.	8 wks, or longer to prevent relapse	2x odds of quitting vs placebo	seizure disorders, bulimia or anorexia, alcohol or sedative withdrawal, MAOIs, pregnancy	insomnia, dry mouth, headache, tremores, nausea, anxiety, seizure
Varenicline	partial agonist for $\alpha_4\beta_2$-nAchR	decreases craving and withdrawal, prevents reinforcing effects of nicotine during lapse to cigarette smoking	1 mg b.i.d PO	12 wks, or up to 24 wks to prevent relapse	4x odds of quitting vs placebo, 2x vs bupropion	pregnancy	nausea, insomnia, headache, neuropsychiatric symptoms

nAchR = nicotinic acetylcholine receptors; SR = sustained-release; b.i.d = twice daily; PO = oral; wks = weeks; MAOI = mono-amine oxidase inhibitor

a cigarette delivers 1-3 mg of nicotine, none of the NRT formulations deliver nicotine at such high doses in a short period of time (Stead *et al.* 2008). Due to this, NRT has low liability for abuse and dependence potential (West *et al.* 2000). The differences in dose and delivery between cigarettes and NRT may also be a reason for lapse or relapse to smoking.

Transdermal patch provides a continuous release of 5, 10 or 15 mg of nicotine over 16 hours or 7, 14 or 21 mg of nicotine over 24 hours, depending on the dose and brand, and does not replace the behavioural activities of smoking or provide much positive reinforcement (Benowitz 2008; Stead *et al.* 2008). The other formulations offer a shorter delivery of nicotine and allow self-titration. Nasal spray is the most rapid-delivery formulation of NRT (1 or 2 0.5 mg doses in each nostril), which provides the most positive reinforcement and fastest relief of withdrawal symptoms (Benowitz 2008; Nides 2008). The inhaler mimicks the behavioural aspects of smoking (cigarette-like device) and delivers 4 mg per puff (Nides 2008). Lozenge and chewing gum come in 2 mg or 4 mg strengths (Stead *et al.* 2008). A meta-analysis suggests that all forms of NRT increase abstinence from smoking by approximately two-fold versus placebo and that there is no difference in efficacy between the formulations (Stead *et al.* 2008). The differences in effectiveness of NRT between men and women is unclear – one meta-analysis of nicotine patch by sex found no differences in efficacy (Munafo *et al.* 2004), while a second meta-analysis of any type of NRT reported a greater decline in long-term efficacy in women versus men, where 12-month cessation remained significant for men but not for women (Cepeda-Benito *et al.* 2004).

NRT is not associated with serious adverse events, and mild side effects are generally formulation-specific – reviewed in Le Foll *et al.* (2007) and Nides (2008). For instance, nicotine patch may cause mild skin irritation at the patch site; gum may cause jaw pain, mouth irritation dyspepsia and hiccoughs; inhaler and lozenge may cause mouth and throat irritation; and spray

may cause runny nose and throat and nasal irritation. NRT is not recommended during pregnancy or acute cardiovascular conditions (McNeil Consumer Healthcare 2008), however NRT is believed to be less harmful to the fetus than tobacco (Dempsey and Benowitz 2001) and studies have suggested safety of NRT in cardiovascular disease (Joseph *et al.* 1996; McRobbie and Hajek 2001; Hubbard *et al.* 2005).

1.3.2 Bupropion

Bupropion is an atypical anti-depressant that also demonstrates efficacy in smoking cessation and decreases nicotine withdrawal symptoms and craving (Jorenby 2002; Hughes *et al.* 2007). It's effect on smoking cessation appears to be mediated by inhibiting the neuronal reuptake of dopamine and norepinephrine (Cryan *et al.* 2003), and may involve its weak antagonism of nicotinic receptors (Fryer and Lukas 1999).

Bupropion is prescribed as an 150 mg sustained-release tablet twice daily (first 3 days is 150 mg once daily) for 8 weeks or possibly longer to prevent relapse (Biovail Pharmaceuticals Canada 2005). Meta-analysis suggested that it doubles the chances of quitting compared to placebo, and produces quit rates similar to NRT (Hughes *et al.* 2007). It can be used as a monotherapy or in combination with NRT. Studies have reported inconsistent results regarding differences in efficacy of bupropion between men and women – some studies have reported no treatment-gender interactions (meta-analysis of short-term outcomes by Gonzales et al. 2002; Scharf and Shiffman 2004), while others suggest women benefit more versus men {Tashkin, 2001 #334}(long-term data analysis by Tashkin *et al.* 2001; Smith *et al.* 2003) or that men benefit more than females (Gonzales *et al.* 2001). Bupropion is equally effective in smokers with or without a history of depression (Hayford *et al.* 1999; Hurt *et al.* 2002; Cox *et al.* 2004).

The most common adverse event is insomnia (30-40% of patients) and dry mouth (10%) (Hughes *et al.* 2007), and the most serious side effect is seizure (0.1% frequency) (Dunner *et al.* 1998; Boshier *et al.* 2003). Due to this risk, bupropion is contraindicated in patients with seizure disorders or at risk of seizure, including bulimia or anorexia, and alcohol or sedative withdrawal (Biovail Pharmaceuticals Canada 2005). Bupropion is also contraindicated in combination with monoamine oxidase inhibitors, and in pregnancy (Biovail Pharmaceuticals Canada 2005).

1.3.3 Varenicline

Varenicline is a partial agonist for neuronal $\alpha_4\beta_2$-nAChRs. It binds with higher affinity but has lower efficacy compared to nicotine, resulting in a moderate and sustained release of dopamine that counteracts the reduced dopamine levels experienced during cessation, thereby relieving nicotine craving and withdrawal symptoms (Coe *et al.* 2005; Rollema *et al.* 2007). Furthermore, by competitively binding to the α4β2-nAChRs, it also protects against nicotine-induced dopaminergic activation if there is a lapse to cigarette smoking (Coe *et al.* 2005; Rollema *et al.* 2007). Therefore, varenicline disrupts the reinforcing effects of nicotine (prevents reward) and counteracts withdrawal.

Varenicline is prescribed as 1 mg twice daily for 12 weeks (1^{st} week involves titration with 0.5 mg once daily for the 1^{st} 3 days and twice daily for the next 4 days) or up to 24 weeks to prevent relapse. 12-week varenicline treatment increases the odds of abstinence by almost 4-fold compared to placebo and almost 2-fold compared to bupropion (Gonzales *et al.* 2002; Jorenby 2002). Long-term abstinence rates and long-term maintenance treatment showed significantly increased quit rates compared to placebo (Tonstad *et al.* 2006; Nakamura *et al.* 2007; Tsai *et al.* 2007). The most common adverse events include nausea (30% of patients), insomnia and headache (Gonzales *et al.* 2006; Jorenby *et al.* 2006). Post-marketing reports have reported

several rare neuropsychiatric symptoms during varenicline therapy, such as depression and suicidal ideation and suicide, and therefore diligent monitoring for neuropsychiatric symptoms, especially in patients with comcomitant psychiatric conditions or with a history of psychiatric symptoms is recommended (Pfizer 2008). Varenicline is not recommended in pregnancy (Pfizer 2008).

1.4 Nicotine Metabolism

1.4.1 Pharmacokinetics

Nicotine from cigarette smoke is rapidly absorbed into plasma and reaches the brain within 10-19 seconds after inhalation (Benowitz 1996). Plasma levels rapidly decay due to widespread distribution (distribution half-life of 15-20 min) and elimination (elimination half-life of 2-3 hours) (Benowitz *et al.* 1982; Jarvis 2004). Nicotine is rapidly and extensively metabolized mainly in the liver and to a smaller extent in the lungs, kidneys, nasal mucosa and brain (Hukkanen *et al.* 2005).

The major metabolic pathway of nicotine metabolism is summarized in Figure 1.1. In humans, approximately 70-80% of nicotine is metabolically inactivated to cotinine (COT) (via C-oxidation), and roughly 90% of this conversion is mediated by the liver cytochromes P450 (CYP) enzyme CYP2A6 (Benowitz and Jacob 1994; Nakajima *et al.* 1996b; Messina *et al.* 1997). COT is subsequently oxidized to form 3'-hydroxycotinine (3HC), which is exclusively catalyzed by CYP2A6 (Nakajima *et al.* 1996a; Dempsey *et al.* 2004). Other CYPs such as CYP2B6 and CYP2D6 can also C-oxidize nicotine, however they have significantly lower affinity for nicotine (10-fold and 12-fold higher Km for CYP2B6 and CYP2D6, respectively) and reduced activity towards COT formation (10% and 5% activity by CYP2B6 and CYP2D6, respectively) compared to CYP2A6 (Nakajima *et al.* 1996b; Messina *et al.* 1997; Yamazaki *et al.*

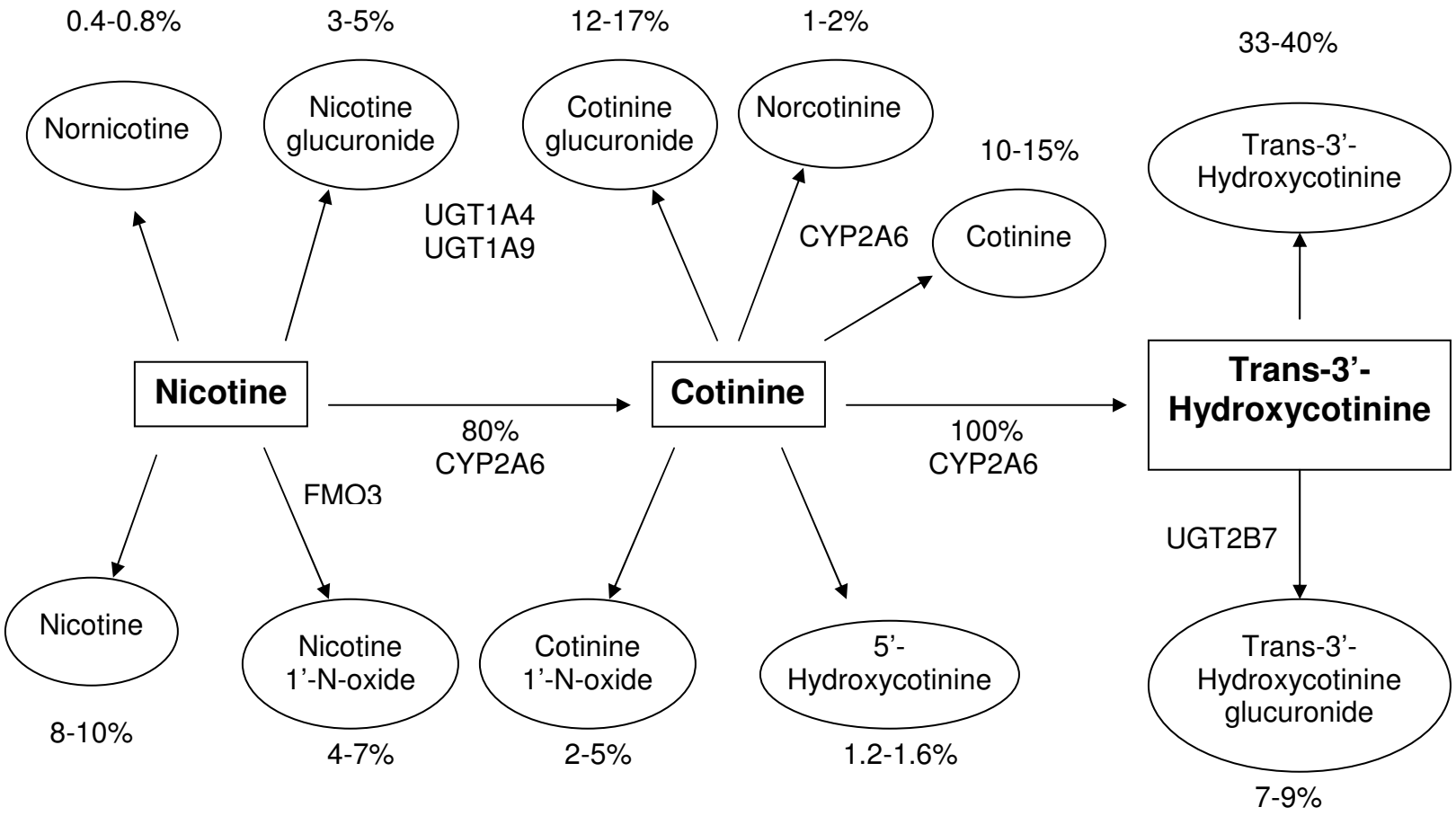


Figure 1.1: Major metabolic pathways of nicotine metabolism. Circled compounds are major metabolites of nicotine excreted in the urine. Percent urinary production associated with each metabolite is indicated (Adapted from Yamanaka *et al.* 2004; Hukkanen *et al.* 2005).

1999). Furthermore, hepatic levels of CYP2B6 are low (Ekins and Wrighton 1999; Yamazaki *et al.* 1999), but CYP2B6 may still play a compensatory role when CYP2A6 activity is substantially reduced (Schoedel *et al.* 2003; Yamanaka *et al.* 2004).

Aside from the main pathway of nicotine metabolism to COT, nicotine is also N-oxidized to nicotine 1'-N-oxide via hepatic flavin-containing monooxygenase 3 (FMO3) and N-demethylated to nornicotine (Benowitz and Jacob 1994; Benowitz *et al.* 1994; Lang *et al.* 1998). Apart from the formation of 3HC from COT, COT is also metabolized to several minor metabolites, including 5'-hydroxycotinine, cotinine 1'-N-oxide and norcotinine (via CYP2A6) (Benowitz *et al.* 1994; Murphy *et al.* 1999). Nicotine, COT and 3HC also undergo glucuronidation. Both nicotine and cotinine are N-glucuronidated by UDP-glucuronosyltransferase (UGT) 1A4 and 1A9 to form nicotine-N-1-β-glucuronide and cotinine-N-1-β-glucuronide, while 3HC is believed to be O-glucuronidated by UGT2B7 to form trans-3'-hydroxycotinine-O-β-D-glucuronide (Kuehl and Murphy 2003; Yamanaka *et al.* 2004; Yamanaka *et al.* 2005).

1.4.2 Nicotine Metabolite Ratio

The conversion of nicotine to COT to 3HC is the major metabolic pathway of nicotine metabolism in humans. COT has a much longer half-life compared to nicotine (14-20 hours versus 2 hours), and therefore plasma COT concentrations remain fairly stable throughout the day and night during smoking and can be used as a marker of nicotine intake (Benowitz *et al.* 1983; Benowitz and Jacob 1994; Zevin *et al.* 1997; Dempsey *et al.* 2004). Furthermore, the half-life of 3HC administered alone is 5-6 hours, but since this elimination half-life is generation-limited (due to longer COT half-life), a ratio of 3HC to COT should remain constant over time (Benowitz and Jacob 2001; Dempsey *et al.* 2004). Since the conversion of COT to 3HC is

entirely due to CYP2A6 activity, the ratio of 3HC/COT is used as a marker of CYP2A6 activity (Neurath 1994; Dempsey *et al.* 2004). Kinetic studies in humans indicate that 3HC/COT is highly correlated with cotinine clearance and cotinine half-life, which further suggests that CYP2A6 is the primary metabolizing enzyme of cotinine (Dempsey *et al.* 2004). Furthermore, 3HC/COT derived from oral nicotine administration and smoking are highly correlated with one another and with oral nicotine clearance, and therefore 3HC/COT can also be used as a marker of nicotine metabolism by CYP2A6 (Dempsey *et al.* 2004). A low 3HC/COT reflects slow CYP2A6 activity and nicotine metabolism, whereas a high 3HC/COT reflects fast CYP2A6 activity and nicotine metabolism.

1.4.3 Interindividual Variability

There is large interindividual and interethnic variation in nicotine metabolism (Benowitz and Jacob 1994; Cholerton *et al.* 1994; Benowitz *et al.* 1995; Benowitz *et al.* 1997; Kwon *et al.* 2001; Nakajima and Yokoi 2005). The source of variability in nicotine metabolism may be due to differences in individual pharmacokinetics, such as urinary pH and liver blood flow (Cholerton *et al.* 1994; Benowitz *et al.* 1997; Hukkanen *et al.* 2005). There is sex-related variation in nicotine metabolism. Estrogen has been found to induce *CYP2A6* transcription *in vitro* (Higashi *et al.* 2007) and female subjects have faster rates of *in vivo* nicotine metabolism compared to males (Benowitz *et al.* 2006a). Variation in CYP2A6 activity can also be due to environmental influences such as diet and medications. For instance, CYP2A6 activity is increased by oral contraceptives (Benowitz et al. 2006), rifampin (Rae *et al.* 2001), dexamethasone (Onica *et al.* 2008) and phenobarbital (Itoh *et al.* 2006). In contrast, CYP2A6 activity can be inhibited by methoxsalen and tryptamine (Kharasch *et al.* 2000), and nicotine (Denton *et al.* 2004).

A significant source of variability in nicotine metabolism has also been attributed to interindividual differences in CYP2A6 levels and activity (Nakajima *et al.* 1996b; Shimada *et al.* 1996; Messina *et al.* 1997). For instance, a 30-fold variation in COT formation via CYP2A6 was observed in human liver microsomes (Messina *et al.* 1997), as well as large variation in *in vitro* conversion of COT to 3HC (Nakajima et al. 1996). As mentioned, there is considerable interethnic variation in nicotine metabolism and CYP2A6 activity. For instance, the level and activity of CYP2A6 is lower in Japanese populations compared to Caucasians (Shimada *et al.* 1996). Black populations appear to have reduced COT clearance compared to Caucasians (Perez-Stable *et al.* 1998), while Chinese-Americans have significantly reduced nicotine and COT clearance when compared to Caucasian- and Latino-Americans (Benowitz et al. 2002).

The interindividual and interethnic variation in CYP2A6 activity is partially attributed to *CYP2A6* genetic polymorphisms and will be discussed below (Nakajima *et al.* 2000; Nakajima *et al.* 2001; Mwenifumbo and Tyndale 2007).

1.5 *CYP2A6* Genetic Variation

1.5.1 CYP2 Gene Cluster

The cytochrome P450 superfamily of mixed-function oxygenases is composed of 18 families, of which the CYP2 family is the largest and most complex (consisting of multiple subfamilies), and one of the most important in the metabolism of xenobiotics (Hoffman *et al.* 2001). The *CYP2* family genes have been found to cluster on human chromosome 19, where loci from the *CYP2A*, *CYP2B* and *CYP2F* subfamilies have been mapped in a 350kb region on 19q12→19q13.2 (Miles *et al.* 1990; Fernandez-Salguero *et al.* 1995; Hoffman *et al.* 1995). Within the CYP2A subfamily, three *CYP2A* genes have been identified and their loci mapped to human chromosome 19: *CYP2A6*, *CYP2A7* and *CYP2A13* (Hoffman *et al.* 2001). A fourth locus

also exists and is a split *CYP2A7* pseudogene, named *CYP2A18* (5' half *CYP2A18*PN and 3' half *CYP2A18PC*) (Hoffman *et al.* 2001). *CYP2A6* shares 97% and 85% exonic sequence identity to *CYP2A7* and *CYP2A13*, respectively (Hoffman *et al.* 2001).

CYP2A6 is a major metabolizing enzyme for nicotine, cotinine and coumarin, and also metabolizes other xenobiotics such as tegafur, methoxyflurane, (+)-*cis*-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride, and tobacco-specific nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Yamazaki *et al.* 1992; Kharasch *et al.* 1995; Nunoya *et al.* 1996; Nunoya *et al.* 1998; Nunoya *et al.* 1999b; Ikeda *et al.* 2000; Komatsu *et al.* 2000; Pelkonen *et al.* 2000; Daigo *et al.* 2002). Although the *CYP2A7* gene produces full-length transcripts in the liver, the enzyme is inactive because it cannot incorporate heme (Yamano *et al.* 1990; Ding *et al.* 1995). CYP2A13 mRNA is expressed in respiratory tissues and the protein is highly active towards tobacco-related nitrosamines, but has low coumarin activity (10% of CYP2A6) (Su *et al.* 2000). However, a study in human liver microsomes revealed large variability in CYP2A6 and CYP2A13 protein levels, but that CYP2A6 levels were significantly higher than CYP2A13 (Zhang *et al.* 2007). Nonetheless, due to the low expression of CYP2A13 in the liver, CYP2A13 is not expected to influence systemic nicotine and COT levels (Koskela *et al.* 1999; Su *et al.* 2000).

1.5.2 *CYP2A6* Genetic Polymorphisms

The expression and activity of CYP2A6 is highly variable, which can partially be explained by the polymorphic nature of *CYP2A6*. Numerous allelic variants and single nucleotide polymorphisms (SNPs) of *CYP2A6* have been discovered and are summarized in http://www.cypalleles.ki.se/cyp2a6.htm. Furthermore, the frequency of *CYP2A6* polymorphisms are known to vary by ethnicity (Schoedel *et al.* 2004; Malaiyandi *et al.* 2005). The impact of

CYP2A6 genetic polymorphisms on CYP2A6 activity, nicotine metabolism and smoking-related behaviours will be outlined below.

1.6 Impact of *CYP2A6* Genotype on Activity and Nicotine Metabolism

Only the most prevalent decrease- and loss-of-function alleles among various ethnic groups will be described below. Therefore, *CYP2A6* genetic variants that have relatively low frequency (*CYP2A6*3, *5, *6, *11, *13, *15*), minimal/unclear impact on CYP2A6 function (*CYP2A6*14*), both low impact and low frequency (*CYP2A6*16, *18, *19, *21*), or are uncharacterized or have limited information on CYP2A6 impact (*CYP2A6*22, *29, *30, *31, *32, *33, *34, *36, *37*) will not be discussed (Yoshida *et al.* 2002; Schoedel *et al.* 2004; Fukami *et al.* 2005b; Al Koudsi *et al.* 2006; Nakajima *et al.* 2006; Mwenifumbo *et al.* 2008). The properties and ethnic frequencies of the described *CYP2A6* genetic variants are summarized in Table 1.2 and Table 1.3. The nomenclature used for all SNPs indicates the genomic position and nature of the nucleotide change(s) (e.g. 1799T>A), followed by the resulting amino acid substitution (e.g. L160H), if any.

1.6.1 Most Prevalent Decrease- and Loss-of-function Alleles

The *CYP2A6*2* allele is due to 1799T>A (L160H), which results in a catalytically inactive CYP2A6 enzyme due to inability to incorporate heme (Yamano *et al.* 1990; Hadidi *et al.* 1997; Oscarson *et al.* 1998). The allele frequency of *CYP2A6*2* is low in Caucasians (2.0-2.2%) and Blacks (0.4-0.9%), and essentially absent in Asians (Rao *et al.* 2000; Kwon *et al.* 2001; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

There are five (A/C, B, D, E, F) *CYP2A6*4* allele variants, which are all *CYP2A6* gene deletions due to an unequal crossover event with *CYP2A7* (Nunoya *et al.* 1999b; Oscarson *et al.*

Table 1.2: Prevalent loss- or decrease-of-function *CYP2A6* genetic variants.▲

***CYP2A6* genetic polymorphisms**				
***CYP2A6* allele**	**Nucleotide changes**	**Protein changes**	**Functional consequence**	**Reference**
**1A*	-	-	normal activity	-
**1x2*	gene duplication (reciprocal of *CYP2A6*4*)		↑ nicotine metabolism *in vivo*	(Rao *et al.* 2000; Benowitz *et al.* 2002a; Fukami *et al.* 2007)
**2*	1799T>A (exon 3)	L160H = cannot incorporate heme	inactive	(Yamano *et al.* 1990; Hadidi *et al.* 1997; Oscarson *et al.* 1998)
**4*	gene deletion (unequal crossover with *CYP2A7*)	no product	inactive	(Nunoya *et al.* 1999; Oscarson *et al.* 1999; Ariyoshi *et al.* 2000; Ariyoshi *et al.* 2002b; Ariyoshi *et al.* 2004; Mwenifumbo *et al.* 2008)
**7*	6558T>C (exon 9)	I471T	↓ coumarin and nicotine activity *in vitro*; ↓ nicotine metabolism i*n vivo*	(Ariyoshi *et al.* 2001; Xu *et al.* 2002; Yoshida *et al.* 2002)
**8*	6600G>T (exon 9)	R485L	No effect on nicotine metabolism *in vivo*	(Ariyoshi *et al.* 2001; Xu *et al.* 2002)
**9*	48T>G in TATA box	↓ protein product (due to ↓ mRNA expression)	↓ nicotine metabolism *in vivo*; ↓ coumarin activity *in vitro*	(Pitarque *et al.* 2001; Kiyotani *et al.* 2003; Yoshida *et al.* 2003; Haberl *et al.* 2005; Benowitz *et al.* 2006b)
**10*	6558T>C 6600G>T (exon 9)	I471T R485L	↓ nicotine metabolism *in vivo*	(Xu *et al.* 2002; Yoshida *et al.* 2002)
**12*	*CYP2A6/CYP2A7* hybrid: exons 1-2 *CYP2A7*, exons 3-9 *CYP2A6*	10 amino acid substitution = ↓ protein	↓ coumarin activity *in vitro*; ↓ coumarin and nicotine metabolism *in vivo*	(Oscarson *et al.* 2002; Benowitz *et al.* 2006b)

**17*	5065G>A (exon 7)	V365M	↓ nicotine metabolism *in vivo*; ↓ nicotine and coumarin activity *in vitro*	(Fukami *et al.* 2004; Ho *et al.* 2008; Mwenifumbo *et al.* 2008)
**20*	2141_2142delAA (exon 4)	frame-shift from codon 196, early stop codon at 220 = truncated protein	↓ nicotine metabolism *in vivo*; ↓ nicotine and coumarin activity *in vitro*	(Fukami *et al.* 2005; Mwenifumbo *et al.* 2008)
**23*	2161C>T (exon 4)	R203C	↓ nicotine metabolism *in vivo*; ↓ nicotine and coumarin activity *in vitro*	(Ho *et al.* 2008; Mwenifumbo *et al.* 2008)
**24*	594G>C (exon 2), 6458A>T (exon 9)	V110L N438Y	↓ nicotine metabolism *in vivo*	(Mwenifumbo *et al.* 2008)
**25*	1672T>C (exon 3)	F118L	↓ nicotine metabolism *in vivo*	(Mwenifumbo *et al.* 2008)
**26*	1672T>C, 1703G>T, 1711T>G, (exon 3)	F118L R128L S131A	↓ nicotine metabolism *in vivo*	(Mwenifumbo *et al.* 2008)
**27*	1672T>C (exon 3), 2162_2163GC>A (exon 4)	F118L R203FS (frameshift)	↓ nicotine metabolism *in vivo*	(Mwenifumbo *et al.* 2008)
**28*	5745A>G, 5750G>C (exon 8)	N418D E419D	↓ nicotine metabolism *in vivo*	(Mwenifumbo *et al.* 2008)
**35*	6458A>T (exon 9)	N438Y	↓ nicotine metabolism *in vitro* and *in vivo*	(Al Koudsi *et al.* 2009)

▲ *CYP2A6* variants that are not described in this table, include variants with: relatively low frequency (*CYP2A6*3, *5, *6, *11, *13, *15*); unclear impact on CYP2A6 function (*CYP2A6*14*); both low impact and low frequency (*CYP2A6*16, *18, *19, *21*); limited information regarding functional impact (*CYP2A6*22, *29, *30, *31, *32, *33, *34, *36, *37*).

Table 1.3: Allele frequencies of prevalent and impactful *CYP2A6* genetic variants in Caucasians, Blacks and Asians. ▲

***CYP2A6* Allele Frequencies**				
***CYP2A6* allele**	**Caucasians**	**Blacks**	**Asians**	**Reference**
**1A*	-	-	-	
**1x2*	1.7%	-	Korean: 0.5%	(Rao *et al.* 2000; Yoshida *et al.* 2002)
**2*	2.0-2.2%	0.4-0.9%	Japanese: 0%; Chinese: 0%; Korean: 0%	(Rao *et al.* 2000; Kwon *et al.* 2001; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**4*	0.13-1.2%	1.9-2.7%	Japanese: 20.1-24.2%; Chinese: 6.7%; Korean: 11.0%	(Rao *et al.* 2000; Kwon *et al.* 2001; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**7*	0%	0%	Japanese: 12.5%; Chinese: 5.7-9.8%; Koreans: 9.4%	(Mwenifumbo *et al.* 2005)
**8*	0%	0%	Japanese: 0%; Chinese: 0%; Koreans: 0%	(Mwenifumbo *et al.* 2005)
**9*	5.2-8.0%	7.2-9.6%	Japanese: 20.3-21.3%; Chinese: 15.6-15.7%; Korean: 22.3%	(Pitarque *et al.* 2001; Yoshida *et al.* 2003; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**10*	0%	0%	Japanese: 3.2%; Chinese: 4.0-4.3%; Koreans: 4.1%	(Mwenifumbo *et al.* 2005)
**12*	2.0-2.2%	0-0.4%	Japanese: 0.8%; Chinese: 0%	(Oscarson *et al.* 2002; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**17*	0%	7.3-9.4%	0%	(Fukami *et al.* 2004; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)

**20*	0%	1.1-1.6%	0%	(Fukami *et al.* 2005a; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**23*	0%	1.1-2.0%	0%	(Ho *et al.* 2008; Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**24*	-	0.7-1.3%	-	(Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**25*	-	0.5-0.9%	-	(Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**26*	-	0.7%	-	(Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**27*	-	0.2-0.7%	-	(Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**28*	-	0.9-2.4%	-	(Mwenifumbo *et al.* 2008; Ho *et al.* 2009)
**35*	0%	2.5-2.9%	0.5-0.8%	(Al Koudsi *et al.* 2009; Ho *et al.* 2009)

▲ *CYP2A6* variants that are not described in this table, include variants with: relatively low frequency (*CYP2A6*3, *5, *6, *11, *13, *15*); unclear impact on CYP2A6 function (*CYP2A6*14*); both low impact and low frequency (*CYP2A6*16, *18, *19, *21*); or limited information regarding functional impact (*CYP2A6*22, *29, *30, *31, *32, *33, *34, *36, *37*).

1999b). *CYP2A7* sequence is found in the 5'-flanking region, with the *CYP2A6* sequence in the 3'-end. A schematic diagram demonstrating the formation of *CYP2A6*4* allele variants is shown in Figure 2.1. The *CYP2A6*4* allele results in absent enzyme activity (Nakajima *et al.* 2000; Nakajima *et al.* 2001).The *CYP2A6*4A/C* variant is composed of *CYP2A7* exons 1-9 and the 3'-untranslated region of *CYP2A6*, due to crossover in the 3'-flanking region of *CYP2A6* (Nunoya *et al.* 1999b; Nunoya *et al.* 1999a; Oscarson *et al.* 1999b; Ariyoshi *et al.* 2000). The *CYP2A6*4B* variant lacks all exons of the *CYP2A6* gene and contains all exons and the 3'-flanking region of *CYP2A7*, due to crossover downstream of both *CYP2A6* and *CYP2A7* (Nunoya *et al.* 1998; Ariyoshi *et al.* 2002; Ariyoshi *et al.* 2004). The *CYP2A6*4D* variant is composed of *CYP2A7* exons 1-8 and *CYP2A6* exon 9 to 3'flanking region, due to crossover in intron 8 or exon 9 (Oscarson *et al.* 1999a). The most recent *CYP2A6*4E* and *CYP2A6*4F* variants have been identified due to extensive sequence variation in both non-coding and coding sequence, and similar to *CYP2A6*4A/C*, they are formed due to crossover in the 3'flanking region of *CYP2A6* (Mwenifumbo *et al.* 2008). The frequency of the *CYP2A6*4* allele is very low in Caucasians (0.13-1.2%) and Blacks (1.9-2.7%), but highly prevalent in Asian populations (6.7%-24.2%) (Rao *et al.* 2000; Kwon *et al.* 2001; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The reciprocal product of the *CYP2A6*4* deletion is the duplication variant *CYP2A6*1x2*, which results in increased nicotine metabolism *in vivo* (Rao *et al.* 2000; Benowitz *et al.* 2002b; Fukami *et al.* 2007). The formation of the *CYP2A6*1x2* duplication variant is shown in Figure 2.1. *CYP2A6*1x2* has an allele frequency of 1.7% in Caucasians and 0.5% in Koreans (Rao *et al.* 2000; Yoshida *et al.* 2002).

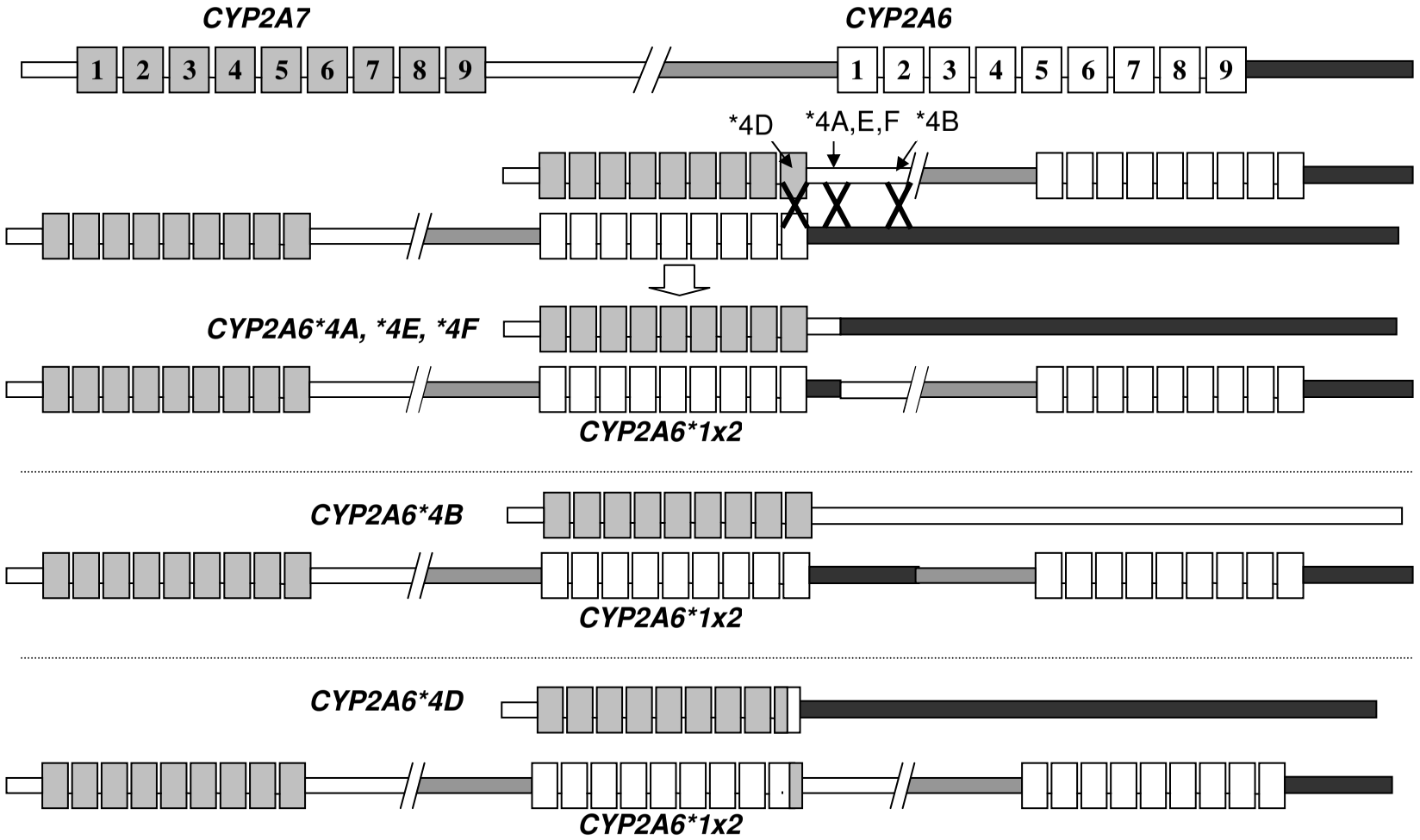


Figure 2.1: A diagram illustrating the formation of *CYP2A6*4* variants and their reciprocal product, *CYP2A6*1x2*. The top schematic illustrates location of *CYP2A6* and *CYP2A7* relative to one another, with numbers indicating exons. *CYP2A6*4* variants are formed when homologous recombination (approximate cross-over junction indicated by **X**) occurs between *CYP2A6* and *CYP2A7*. Crossover in the 3'-flanking region of *CYP2A6* results in *CYP2A6*4A/C, CYP2A6*4E or CYP2A6*4F;* crossover in the downstream region of *CYP2A6* and *CYP2A7* results in *CYP2A6*4B*; and crossover in intron 8/exon 9 results in *CYP2A6*4D*.

The *CYP2A6*9* variant has a -48T>G in the TATA box of the 5'-flanking region of *CYP2A6* (Pitarque *et al.* 2001). This results in reduced transcriptional activity, decreased expression of CYP2A6 mRNA in human liver and decreased production of CYP2A6 protein (Kiyotani *et al.* 2003). Consequently, *in vitro* coumarin activity (Kiyotani *et al.* 2003; Yoshida *et al.* 2003; Haberl *et al.* 2005) and *in vivo* nicotine metabolism are reduced (Yoshida *et al.* 2003; Benowitz *et al.* 2006b). The *CYP2A6*9* allele is highly prevalent in Caucasians (5.2-8.0%), Blacks (7.2-9.6%), and Asians (15.6-22.3%) (Pitarque *et al.* 2001; Yoshida *et al.* 2003; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The *CYP2A6*12* allele is a *CYP2A7/CYP2A6* hybrid allele, composed of *CYP2A7* exons 1-2 and *CYP2A6* exons 3-9, due to unequal crossover between *CYP2A6* and *CYP2A7* in intron 2 (Oscarson *et al.* 2002). The resulting protein has 10 amino acid substitutions. The *CYP2A6*12* allele reduces CYP2A6 protein levels, and results in reduced coumarin activity *in vitro* and *in vivo* (Oscarson *et al.* 2002) and reduced nicotine metabolism *in vivo* (Benowitz *et al.* 2006b). The allelic frequency of *CYP2A6*12* is 2.0-2.2% in Caucasians, 0-0.4% in Blacks and 0-0.8% in Asians (Oscarson *et al.* 2002; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Mwenifumbo *et al.* 2008; Ho *et al.* 2009). The identification of several additional SNPs in *CYP2A6*12* led to the characterization of *CYP2A6*12B* (1620T>C in intron 2*)* and *CYP2A6*12C* (1620T>C and 1630T>C in intron 2) variants, however the contribution of these additional SNPs to CYP2A6 levels or activity are unknown (Haberl *et al.* 2005).

1.6.2 Decreased/Loss-of-function Alleles in Black Populations

The *CYP2A6*17* allele has a 5065G>A (V365M) which results in significantly decreased coumarin and nicotine activity *in vitro* and decreased nicotine metabolism *in vivo* (Fukami *et al.* 2004; Ho *et al.* 2008). *CYP2A6*17* occurs at a frequency of 7.3-9.4% in Black populations, but

is absent in Caucasians, Japanese and Koreans (Fukami *et al.* 2004; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The *CYP2A6*20* allele has a deletion of two nucleotides 2141_2142delAA, which results in a frame-shift from codon 196 and an early stop codon at 220, producing a truncated CYP2A6 protein (Fukami *et al.* 2005a). *CYP2A6*20* reduces coumarin and nicotine activity *in vitro* (Fukami *et al.* 2005a) and nicotine metabolism *in vivo* (Fukami *et al.* 2005a; Mwenifumbo *et al.* 2008). The allelic frequency of *CYP2A6*20* is 1.1-1.6 % in Black populations, but is absent in Caucasians and Asians (Fukami *et al.* 2004; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The *CYP2A6*23* variant contains a 2161C>T (R203C), resulting in reduced coumarin and nicotine activity *in vitro* (Ho *et al.* 2008) and nicotine metabolism *in vivo* (Ho *et al.* 2008; Mwenifumbo *et al.* 2008). This allele has a frequency of 1.1-2.0% in Black populations, but is not detected in Caucasians or Asians (Ho *et al.* 2008; Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The *CYP2A6*24* variant contains 594G>C (V110L) and 6458A>T (N438Y), resulting in a protein that exhibits reduced nicotine metabolism *in vivo* (Mwenifumbo *et al.* 2008). The allelic frequency is 0.7-1.3% in Black populations (Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

The *CYP2A6*25, *26* and *27* variants all share the non-synonomous SNP 1672T>C (F118L) (Mwenifumbo *et al.* 2008). *CYP2A6*25* contains only 1672T>C, while *CYP2A6*26* contains 1672T>C as well as 1703G>T (R128L) and 1711T>G (S131A), and *CYP2A6*27* contains 1672T>C and 2162_2163GC>A (R203FS, frame-shift). The *CYP2A6*25, *26* and *27* variants all decrease nicotine metabolism *in vivo,* and have an allelic frequency of 0.5-0.9%, 0.7%, and 0.2-0.7% in Black populations, respectively (Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

*CYP2A6*28A* contains two SNPs, 5745A>G (N418D) and 5750G>C (E419D), while *CYP2A6*28B* is identical to *CYP2A6*28A* with the additional insert 6960_6961insGAAAAG in the 3'-flanking region (Mwenifumbo *et al.* 2008). Individuals with either *CYP2A6*28* allele have compromised nicotine metabolism, and the allele occurs at a frequency of 0.9-2.4% in Black populations (Mwenifumbo *et al.* 2008; Ho *et al.* 2009).

*CYP2A6*35* contains the SNP 6458A>T (N438Y) which results in decreased nicotine metabolism *in vitro* and *in vivo* (Al Koudsi *et al.* 2009). *CYP2A6*35* occurs at an allelic frequency of 2.5-2.9% in Black populations, 0.5-0.8% in Asians, and is not found in Caucasians (Al Koudsi *et al.* 2009; Ho *et al.* 2009).

1.6.3 Decreased/Loss-of-function Alleles in Asian Populations

In addition to *CYP2A6*4* and *CYP2A6*9*, there are several other variants common among Asian ethnicities. *CYP2A6*7* contains 6558T>C (I471T), which abolishes nicotine and coumarin metabolism *in vitro* (Ariyoshi *et al.* 2001; Xu *et al.* 2002). *In vivo* studies indicate that the *CYP2A6*7* allele reduces nicotine metabolism (Xu *et al.* 2002; Yoshida *et al.* 2002). *CYP2A6*7* has an allelic frequency of 12.5% in Japanese, 5.7-9.8% in Chinese, 9.4% in Koreans, but is absent in Blacks and Caucasians (Mwenifumbo *et al.* 2005). *CYP2A6*8* contains 6600G>T (R485L), but does not alter nicotine metabolism i*n vivo* (Ariyoshi *et al.* 2001; Xu *et al.* 2002). The *CYP2A6*8* allele is essentially absent in Japanese, Chinese, Korean, Black and Caucasian populations (Mwenifumbo *et al.* 2005). However, *CYP2A6*8* variation has an impact when it is in a haplotype with *CYP2A6*7* (on the same allele), termed *CYP2A6*10*. Individuals with *CYP2A6*10* have reduced nicotine metabolism (Xu *et al.* 2002; Yoshida *et al.* 2002). *CYP2A6*10* has an allele frequency of 3.2% in Japanese, 4.0-4.3% in Chinese, 4.1% in Koreans, but is absent in Blacks or Caucasians (Mwenifumbo *et al.* 2005).

1.6.4 Genotype Grouping Strategy

On the basis of each *CYP2A6* allele's established impact on nicotine metabolism *in vivo* (loss-of-function or decrease-of-function), individuals are placed in one of three *CYP2A6* genotype groups (normal, intermediate, or slow metabolizer) according to their *CYP2A6* genetic variation (Table 1.4) (Schoedel *et al.* 2004; Malaiyandi *et al.* 2005; Benowitz *et al.* 2006b; Ho *et al.* 2009). These genotype groups differ in CYP2A6 activity measured by 3HC/COT, where 3HC/COT is significantly higher in normal metabolizers compared to slow metabolizers (Benowitz *et al.* 2006b; Malaiyandi *et al.* 2006).

The slow metabolizer (SM) group refers to individuals hypothesized to have 50% or less of normal CYP2A6 activity, due to possession of at least one loss-of-function allele (*CYP2A6*2,*4, *7, *10, *17, *20, *23, *24, *25, *26, *27* or **35*), or possession of two decrease-of-function alleles (*CYP2A6*9* or **12*), or any combination of a loss-of-function allele with a decrease-of-function allele (Malaiyandi *et al.* 2005; Benowitz *et al.* 2006b; Ho *et al.* 2009). The intermediate metabolizer (IM) group refers to individuals hypothesized to have 50-75% CYP2A6 activity, due to possession of one decrease-of-function allele. The normal metabolizer (NM) group refers to individuals hypothesized to have normal CYP2A6 activity, due to the absence of any detected loss-of-function or decrease-of-function alleles. Although *CYP2A6*28* has been assumed to decrease CYP2A6 activity *in vivo*, individuals with this allele have previously been excluded from *CYP2A6* genotype grouping because of the large variation in 3HC/COT values reducing the ability to group it with confidence (Ho *et al.* 2009).

1.7 Impact of *CYP2A6* Genotype on Smoking

Since the pattern of smoking and cravings for cigarettes are related to nicotine levels in the

Table 1.4: *CYP2A6* genotype groupings.

Metabolizer group	CYP2A6 activity	*CYP2A6* genotype
Slow Metabolizer (SM)	≤50%	≥1 loss-of-function allele: *CYP2A6*2,*4,*7,*10,*17,*20,*23,*24,*25,*26,*27, or *35* or 2 decrease-of-function alleles: *CYP2A6*9 or *12* or Combination of loss-of-function and decrease-of-function allele
Intermediate Metabolizer (IM)	~75%	1 decrease-of-function allele: *CYP2A6*9 or *12*
Normal Metabolizer (NM)	100%	No loss-of-function or decrease-of-function alleles

brain (McMorrow and Foxx 1983; Benowitz and Jacob 1985; Zacny and Stitzer 1988; Djordjevic *et al.* 1995; Scherer 1999; Jarvik *et al.* 2000; Kassel *et al.* 2007), and since CYP2A6 is the main enzyme that removes nicotine from the blood, genetic variation in the rate and extent of nicotine inactivation by CYP2A6 may alter risk for dependence, smoking behaviours, and likelihood of quitting.

1.7.1 Smoking Behaviours

Studies suggest that SMs smoke less intensely compared to NMs. For instance, SMs smoke fewer cigarettes per day (CPD) and take smaller puffs (i.e. puff volume) compared to NMs (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Strasser *et al.* 2007). However, not all studies have found *CYP2A6* associations with CPD (Loriot *et al.* 2001; Ho *et al.* 2009).

1.7.2 Smoking Status

CYP2A6 genotype associates with the likelihood of being a smoker. Although SMs have a significantly lower age of first smoking compared to NMs, they trend towards smoking for a shorter duration (Schoedel *et al.* 2004). Studies suggest that SMs are less likely to be current smokers. One study in Caucasians showed that the proportion of SMs (based on *CYP2A6*2, *4, *9,* and **12* genotypes) was significantly lower in current smokers (DSM-IV dependent and non-dependent) compared to non-smokers (Schoedel *et al.* 2004). A separate study similarly found that the frequency of the *CYP2A6*4* allele was higher and *CYP2A6*1A/1B* was lower in non-smokers compared to smokers (Iwahashi *et al.* 2004).

1.7.3 Dependence

Studies in adolescents suggest that *CYP2A6* genotype also associates with dependence. One study found that adolescent SMs have an increased risk of becoming dependent, but smoke less

once dependent (O'Loughlin *et al.* 2004), while another study suggested that adolescent SMs progress in nicotine dependence at a slower rate and plateau more quickly compared to NMs (Audrain-McGovern *et al.* 2007).

However, smoking cessation studies in adults suggest no difference in the level of nicotine dependence between SMs, IMs and NMs (Malaiyandi *et al.* 2006; Ho *et al.* 2009). Similarly, in a smoking population, the proportion of CYP2A6 SMs was similar between non-dependent smokers and DSM-IV dependent smokers (Schoedel *et al.* 2004).

1.7.4 Cessation

CYP2A6 genotype appears to influence cessation. One retrospective study suggested that individuals possessing a *CYP2A6*2* allele had a 1.75-fold higher likelihood of quitting smoking compared to those with no *CYP2A6*2* allele (Gu *et al.* 2000). *CYP2A6* genotype also influences usage of NRT and quit rates. For instance, in one NRT clinical trial, SMs (based on *CYP2A6*2, *4, *9,* and **12* genotypes) had higher nicotine plasma levels obtained with nicotine patch compared to NMs, while among those given nicotine spray, SMs obtained similar nicotine levels as NMs by using fewer doses of nicotine spray/day (Malaiyandi *et al.* 2006). This suggests that *CYP2A6* genotype influences self-titration of NRTs such as nicotine spray. In this study by Malaiyandi *et al.* (2006), the impact of *CYP2A6* genotype on success of quitting with nicotine patch and spray could not be determined due to the low number of genetically identified SMs. However, another nicotine gum trial in African-American light smokers found that smokers in the slowest *CYP2A6* genotype group (based on *CYP2A6*2, *4, *9, *12, *17, *20, *23, *24, *25, *26, *27, and *35* genotypes*)* tended to have higher quit rates (7-day point prevalence abstinence, verified by exhaled carbon monoxide levels <10ppm) compared to NMs and IMs (26% vs 21% at end-of-treatment (EOT), 22% vs 20% at 6-month follow-up) (Ho *et al.* 2009).

1.8 Impact of 3HC/COT Metabolite Ratio on Smoking and Cessation

Similar to *CYP2A6* genotype, the 3HC/COT ratio correlates significantly with CPD, where slow metabolism (i.e. low 3HC/COT) is associated with decreased CPD compared to fast metabolism (Benowitz *et al.* 2003; Lerman *et al.* 2006; Schnoll *et al.* 2009). In addition, similar to the effect of *CYP2A6* genotype on nicotine dependence in adult smokers, 3HC/COT is not significantly correlated with nicotine dependence scores (Lerman *et al.* 2006; Schnoll *et al.* 2009).

Like *CYP2A6* genotype, slow metabolism measured by the 3HC/COT ratio is also associated with higher quit rates with NRT. For instance, 3HC/COT predicts the effectiveness of nicotine patch treatment for smoking cessation (7-day point prevalence abstinence, verified by breath carbon monoxide <10ppm), where the quartile of smokers with the lowest 3HC/COT ratios had higher odds of abstinence (~46% at EOT and ~30% at follow-up) compared to the quartile of smokers with the highest 3HC/COT (~28% at EOT and <1% at follow-up) (Lerman *et al.* 2006). These findings were validated by Schnoll *et al.* (2009), where smokers in the lowest 3HC/COT quartile had significantly higher EOT quit rates compared to smokers in the upper 3 quartiles (42% vs 28%). Similarly, a placebo-controlled NRT trial with nicotine gum in African-American light smokers found that smokers in the lowest 3HC/COT quartile had higher quit rates (29% at EOT and 27% at follow-up) compared to smokers in the upper 3 quartiles (21% at EOT and 19% at follow-up) (Ho *et al.* 2009).

Similar effects are seen in placebo treatments arms of smoking cessation studies. For instance, in a placebo-controlled bupropion trial, smokers in the lowest 3HC/COT quartile had significantly higher quit rates (32% at EOT and 19% at follow-up) with placebo compared to smokers in the highest 3HC/COT quartile (10% at EOT and 8% at follow-up) (Patterson *et al.*

2008). However, unlike with nicotine patch and gum, quit rates with bupropion do not differ between slow (32% at EOT and 25% at follow-up) and fast metabolizers (34% at EOT and 27% at follow-up) (Patterson *et al.* 2008). Therefore, smokers in the highest 3HC/COT quartile benefit the most from bupropion treatment compared to placebo (34% vs 10% abstinence rate at EOT), whereas smokers in the lowest quartile have equivalent quit rates with placebo and bupropion (32% at EOT).

1.9 Statement of Problem

The goal of this study was to address the limited information regarding direct comparisons in efficacy between various durations of nicotine patch therapy and the effect of *CYP2A6* genotype on quit rates with nicotine patch. The primary aims of this study were:

1. To directly compare the efficacy of 8-week nicotine patch therapy (followed by 16-week placebo patch) versus 24-week nicotine patch therapy on end-of-treatment (EOT, at 24 weeks) and long-term abstinence (at 52 weeks after quit date).
2. To determine the effect of *CYP2A6* genotype on efficacy of nicotine patch therapy.
3. To directly compare 3HC/COT and *CYP2A6* genotype as predictors of abstinence on patch therapy.

These primary aims will be discussed below with an explanation of the rationale behind each aim, followed by the objective and hypotheses.

1.10 Rationales, Objectives and Hypotheses

Primary Aim #1

Rationale

Although indirect comparisons between 8-week NRT and longer-duration NRT suggest that there is no difference in efficacy for smoking cessation (Stead *et al.* 2008), the best study design to test this hypothesis is to directly compare treatment durations in a single clinical trial. However, the number of published studies directly comparing various durations of NRT is limited. There is just one study to note, which was a large multi-centered study in Europe comparing 8-week versus 22-week nicotine patch therapy, and found no difference in long-term abstinence (Tonnesen *et al.* 1999). However, thus far this type of study has not been replicated. Two other smaller studies comparing 3-week versus 12-week patch therapy (Bolin *et al.* 1999) and 3-week versus 6-week patch therapy (Glavas and Rumboldt 2003) also did not find any differences in abstinence rates. However, the clinical application of these two studies is limited, considering the standard treatment time of 8 weeks for NRT. Therefore, there is a demand for a repeat in clinical trials directly comparing standard 8-week NRT to a longer duration of treatment with a further analysis of the role of CYP2A6 in the outcomes.

Objective

To compare efficacy of 8-week nicotine patch (followed by 16 week placebo patch) versus 24-week nicotine patch in a randomized double-blinded clinical trial for smokers wishing to quit. EOT (at 24 weeks) and long-term abstinence (52 weeks after quit date) will be used to make comparisons.

Hypothesis

1) Quit rates with 24-week nicotine patch therapy will be higher than quit rates with 8-week nicotine patch therapy at EOT (24 weeks) and follow-up (52 weeks).

   Reasoning for Hypothesis 1: At EOT (24 weeks), smokers receiving 24-week nicotine patch had immediately completed their patch treatment, whereas smokers receiving 8-week nicotine patch had just completed their successive 16-week placebo phase. The relapse rate during the 16-week placebo phase after 8-week patch is expected to be higher than the concurrent time during 24-week patch, due to emergence of nicotine withdrawal and craving symptoms.

   Long-term abstinence is expected to be higher for the 24-week versus 8-week treatment because a longer duration of nicotine patch therapy allows for increased smoke-free nicotine treatment time for adjusting to reduced nicotine levels, adjusting to the absence of behavioural aspects of smoking, and learning to control cravings and urges before the nicotine therapy is completed at end-of-treatment. Also, smoking cessation studies have shown that nicotine patch significantly reduces the risk of progression of an initial lapse to relapse (Shiffman *et al.* 2006a; Shiffman *et al.* 2006b). Therefore, a 24-week versus 8-week treatment time will provide a longer time-frame for potentially reducing the progression of smoking lapses during therapy into full relapse, thereby increasing quit rates.

Primary Aim #2

Rationale

Slow metabolizers, defined by low 3HC/COT, have higher quit rates with patch (Lerman *et al.* 2006; Schnoll *et al.* 2009), nicotine gum (Ho *et al.* 2009), and placebo (Patterson *et al.* 2008; Ho *et al.* 2009) compared to fast metabolizers. Since *CYP2A6* genotype associates with

3HC/COT, where those with SM genotype have lower 3HC/COT compared to fast *CYP2A6* metabolizers (Malaiyandi *et al.* 2006), perhaps *CYP2A6* genotype may also associate with abstinence on NRT. *CYP2A6* genotype also associates with smoking behaviour, such that SMs smoke less intensely, smoke for shorter durations, and are less likely to be current smokers (Rao *et al.* 2000; Ariyoshi *et al.* 2002b; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Strasser *et al.* 2007). Therefore, if *CYP2A6* genotype is a predictor of smoking behaviour, perhaps it can also predict the ability to quit. A previous study by Malaiyandi *et al.* (2006) aimed to assess the association of *CYP2A6* genotype with efficacy of nicotine spray and patch, but the study was under-powered (particularly in the number of SMs) to detect an effect. Therefore, the effect of *CYP2A6* genotype on abstinence with nicotine patch in a large smoking population remains undetermined.

Aside from the association of *CYP2A6* genotype with 3HC/COT, there are other similarities between the impact of 3HC/COT and *CYP2A6* genotype on smoking behaviour and cessation. For instance, similar to smokers with low 3HC/COT (Benowitz *et al.* 2003), smokers with SM genotype smoke fewer CPD (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006). In addition, similar to the higher quit rates seen among smokers with low 3HC/COT, smokers with SM genotype had increased likelihood of quitting in a retrospective study and trended towards higher quit rates on nicotine gum in an NRT smoking cessation trial, compared to NMs (Gu *et al.* 2000; Ho *et al.* 2009). However, aside from the latter study in such a specialized population (i.e. African-American light smokers) there have been no published NRT clinical trials studying the association of *CYP2A6* genotype with smoking cessation within a general smoking population. In addition, no studies have been done with nicotine patch therapy, which is the most commonly used form of NRT.

Objective

To establish if and how *CYP2A6* genetic variation impacts the efficacy of standard 8-week nicotine patch treatment and extended 24-week nicotine patch treatment in smokers. Efficacy will be assessed by abstinence at EOT (24 weeks) and follow-up (52 weeks).

Hypotheses

2) Smokers with SM genotype will have better quit rates on standard 8-week nicotine patch treatment at EOT (24 weeks) and follow-up (52 weeks), compared to NMs.
3) Smokers with SM genotype will have better quit rates on extended 24-week nicotine patch treatment at EOT (24 weeks) and follow-up (52 weeks), compared to NMs.

   Reasoning for Hypothesis 2 and 3: Studies suggest that smokers with SM genotype have increased ability to quit versus NMs (Gu *et al.* 2000; Ho *et al.* 2009). It also appears that SM genotype decreases the intensity, duration and likelihood of smoking (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Strasser *et al.* 2007), which may translate to more successful quitting. This decreased intensity and duration of smoking also leads to decreased brain exposure to nicotine, and perhaps decreased development of brain neuroadaptations and tolerance, which may increase the ability to quit.
4) All *CYP2A6* genotype groups will have better quit rates on 24- versus 8-week nicotine patch treatment at EOT (24 weeks) and follow-up (52 weeks), with the largest gain occurring in the NMs.

   Reasoning for Hypothesis 4: If NMs smoke more intensely, for longer durations, and perhaps have increased neuroadaptations and tolerance compared to SMs, longer duration of NRT may be required to overcome the behavioural aspects of smoking and treat the increased neurological changes in this group compared to SMs.

Primary Aim #3

Rationale

Studies suggest that the 3HC/COT ratio is a good predictor of quit rates on placebo, nicotine patch and gum, and bupropion, where slow metabolizers have increased quit rates on placebo and nicotine patch and gum, and fast metabolizers benefit the most from bupropion treatment versus placebo (Lerman *et al.* 2006; Patterson *et al.* 2008; Ho *et al.* 2009; Schnoll *et al.* 2009). Therefore, 3HC/COT may be a useful tool in personalizing smoking cessation therapies for smokers. Although *CYP2A6* genotype associates with 3HC/COT (Malaiyandi *et al.* 2006), there is a gap in knowledge with respect to the potential predictive ability of *CYP2A6* genotype on quit rates with smoking cessation therapies. Assessing the potential of *CYP2A6* genotype as a predictor of abstinence with therapy is worth pursuing because using genotypic measures has various advantages over using phenotypic measures (e.g. 3HC/COT) in clinical and research settings.

For instance, genotyping can be performed once and used as a reference throughout the course of an individual's lifetime. Furthermore, unlike phenotype, an individual's genotype is not subject to variation induced by the environment such as diet or medications. With respect to CYP2A6 activity for example, individuals on medications such as oral contraceptives (Benowitz *et al.* 2006a) or dexamethasone (Onica *et al.* 2008) will have increased CYP2A6 activity, while exposure to inhibitors such as methoxsalen will decrease CYP2A6 activity (Kharasch *et al.* 2000). Therefore, a phenotypic measure is perhaps a better reflection of an individual's current CYP2A6 activity compared to *CYP2A6* genotype. However, an individual's exposure to environmental influences on CYP2A6 function may change over time, and therefore *CYP2A6* genotype may be a more reliable measure of an individual's CYP2A6 activity. Since genotyping

is a qualitative measure, it is also subject to reduced variation/errors that occur during quantitative phenotyping measures due to differences in equipment, methodology, and handling. Genotyping is also advantageous with regards to usage – DNA can be shipped between labs and facilities, and/or be kept frozen for long durations and used later. A single DNA sample can be used numerous times for various genotyping assays.

Objective

To assess the relative merit of *CYP2A6* genotype versus 3HC/COT as a predictive measure for smoking cessation.

Hypothesis

5) *CYP2A6* genotype will be a significant predictor of abstinence rates with nicotine patch therapy, but this predictive quality may be weaker compared to that obtained with 3HC/COT.

   Reasoning for Hypothesis 5: In an NRT clinical trial in Caucasians conducted by Lerman *et al.* (2006), 3HC/COT predicted the effectiveness of 8-week nicotine patch therapy at EOT (8 weeks) and 6-month follow-up. However, the impact of *CYP2A6* genotype on success of quitting with patch in this study could not be determined, primarily due to the low number of genetically identified SMs (Malaiyandi *et al.* 2006). In a smoking cessation trial with nicotine gum in African-American light smokers, individuals with low 3HC/COT had significantly higher quit rates than those with high 3HC/COT, while smokers with SM genotype trended towards higher quitting rates (but did not reach significance) (Ho *et al.* 2009). Therefore, previous studies suggest 3HC/COT is a good predictor of quit rates with nicotine patch and nicotine gum, while *CYP2A6* genotype may also be a good predictor of quit rates, but not as strong as 3HC/COT.

2. MATERIALS & METHODS

2.1 Study Overview

This study was part of an open-label transdermal NRT clinical trial lead by Dr. Caryn Lerman at the University of Pennsylvania. The study was supported by the National Institutes of Health, protocol number 801851. The Health Sciences Research Ethics Board of the University of Toronto also approved this study (#20284). Dr. Caryn Lerman conducted participant recruitment, clinical trial management, data and sample collection and preparation. DNA extraction was performed at the University of Pennsylvania and DNA samples were sent to Dr. Rachel Tyndale's lab for *CYP2A6* genotyping. Plasma metabolites were measured at the University of California San Francisco by Dr. Neal Benowitz.

2.2 Study Design

2.2.1 Subject Recruitment and Screening

Subjects were recruited via advertisements for a free smoking cessation program at the University of Pennsylvania, and enrolment took place Oct.1, 2004 – Jan.31, 2008. Male and female smokers aged 18-65, who smoked >10 CPD and were interested in smoking cessation were eligible for the study. Exclusion criteria included female subjects that were pregnant/planning/lactating; individuals with contraindications for transdermal nicotine (uncontrolled hyptertension, liver/kidney failure in last 6 months), receiving cancer treatment or diagnosed with cancer in the last 6 months, diagnosed with a DSM-IV substance use disorder (alcohol, cocaine, marijuana or stimulants, benzodiazepines), currently using NRT or concomitant medications (e.g. monoamine oxidase inhibitors within past 14 days, antipsychotics, endogenous steroids, stimulants, antidepressants, including wellbutrin or bupropion), or currently

diagnosed with an Axis I psychiatric disorder (i.e. psychosis, current major depression, current mania).

2.2.2 Protocol

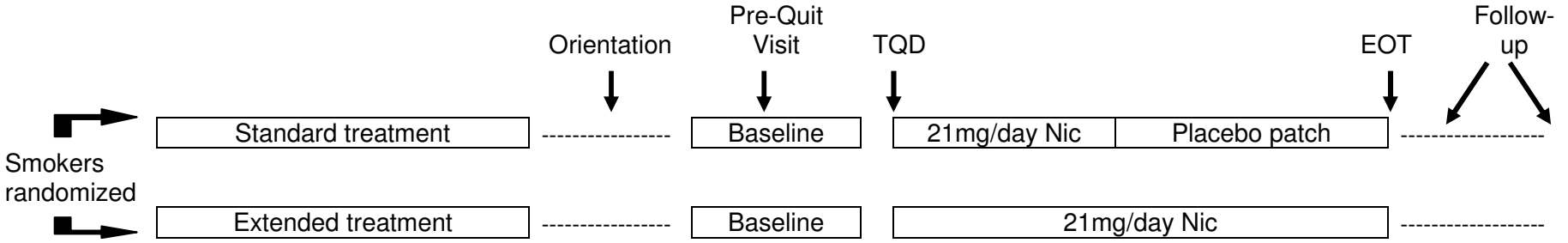
Smokers (n=576) were randomized to one of two treatment arms with open-label transdermal NRT (Nicoderm CQ; GlaxoSmithKline, Research Triangle Park, North Carolina, USA): standard treatment (21 mg/24 hours nicotine patch for 8 weeks, followed by placebo patch for 16 weeks) or extended treatment (21 mg/24 hours nicotine for 24 weeks). Smokers received a full-dose therapy throughout treatment because there is no difference in efficacy compared to tapered-dose therapy (Stapleton *et al.* 1995). A schematic of the study protocol is shown in Table 2.1. All subjects attended a pre-quit counselling session at baseline (week -2), set their target quit date for week 0, and received further behavioural counselling sessions throughout their 24-week treatment. These counselling sessions were based on standard smoking cessation behavioural treatment (Lerman *et al.* 2004; Fiore *et al.* 2008). Subjects were followed-up at 28 weeks and 52 weeks after target quit date.

2.2.3 Assessments

2.2.3.1 Eligibility Screening Variables and Covariates

At baseline, subjects underwent a medical history and physical examination, and were asked for self-reported demographic measures (age, race, sex, marital status and education) and smoking behaviour (age at smoking initiation, CPD, number of previous quit attempts, length of prior abstinence periods, previous use of NRT). Subjects were also administered the FTND, which is a 6-item questionnaire used to assess nicotine dependence (Heatherton *et al.* 1991). Weight was measured at baseline and monitored throughout treatment and at follow-up sessions.

Table 2.1: A Schematic of the study protocol and timeline of assessments.



STUDY WEEK	-4	-2	0	1	4	8	12	16	20	24	28	48
Counselling		X	X	X	X	X	X	X	X			
Screening Variables and Covariates												
Weight		X	X	X	X	X	X	X	X	X	X	X
Demographics, smoking history, psych and medical history, inattention and hyperactive symptoms		X										
Depression symptoms		X								X	X	X
Anger		X		X								
Medication expectations												X
Genotype		X										
Mediating Variables												
Withdrawal, smoking Urge/Craving, positive and negative affect		X	X	X	X	X	X	X	X	X		
Treatment Variables												
Nicotine/cotinine/3HC		X		X								
Usage, side-effects			X	X	X	X	X	X	X	X		
Cost-effectiveness			X	X	X	X	X	X	X	X	X	X
Smoking Outcomes												
Cessation/smoking rate		X		X		X				X	X	X

TQD = target quit date; EOT = end-of-treatment; Nic = nicotine

Current psychiatric disorders were assessed using the Structured Clinical Interview for DSM-IV, Non-patient Edition (SCID-NP) (Spitzer R 1990) over telephone during eligibility screening, and subjects meeting the criteria for Axis 1 disorders were excluded from participation. Attention-deficit hyperactivity disorder (ADHD) symptoms from DSM-IV were assessed at baseline using the 18-item Current Symptoms Scale, Self Report Form (Kessler *et al.* 2005). Depression was assessed at baseline, EOT (24 weeks) and at follow-up sessions with the 20-item Likert-format Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977). Anger was assessed at baseline and week 1 with Spielberger's 15-item self-report scale (Spielberger 1999).

At baseline, and throughout treatment, withdrawal symptoms associated with smoking cessation were assessed using the 18-item Minnesota Nicotine Withdrawal Scale (Hughes and Hatsukami 1986), smoking urges and craving for cigarettes were assessed with a 10-item Questionnaire on Smoking Urges (QSU) (Tiffany and Drobes 1991), and positive and negative effect was assessed with a 20-item Likert-format self-report measure, called the Positive and Negative Affect Schedule (Watson *et al.* 1988).

2.2.3.2 Treatment Variables

Plasma nicotine, COT and 3HC were measured at baseline (while still smoking) and week 1 (among participants with confirmed abstinence). Blood samples were drawn at the University of Pennsylvania and sent to Dr. Benowitz at the University of California for metabolite measures. Nicotine was measured via gas chromatography with nitrogen-phosphorous detection (Jacob *et al.* 1981), and COT and 3HC via liquid chromatography with tandem mass spectrometry (Dempsey *et al.* 2004).

Patch use was assessed throughout treatment via self-report and by returning used patches each week for patch counts. COT levels were used as a secondary measure of overall usage in

those claiming abstinence. Patch-related side effects were assessed throughout treatment with a checklist for headaches, nausea, vomiting, constipation-diarrhea, sleep problems, rash, skin reactions, etc. Symptom severity was rated from 0 (none) to 3 (severe), and a total side-effect score was computed.

2.2.3.3 Smoking Outcomes

Cessation/smoking rate was assessed and verified (self-report cessation, verified by 7-day point prevalence breath carbon monoxide sample ≤10 ppm) at baseline (SRNT, 2002), during treatment phase, end-of treatment, and at follow-up visits. Subjects that self-reported cessation and provided a sample of breath carbon monoxide ≤10 ppm were considered abstinent. Subjects who withdrew from the trial, or failed to provide a carbon monoxide breath sample, or provided a breath sample >10 ppm were considered smokers.

***2.3 CYP2A6* Genotyping**

2.3.1 Overview

DNA extraction was performed at the University of Pennsylvania and DNA samples were sent to Dr. Tyndale's lab and stored at -20 ^{o}C. We received 570 DNA samples composed of 482 Caucasians, 84 Blacks, and 4 Asians. *CYP2A6* genotyping was successfully completed for 557 samples.

Genotyping assays were used to detect *CYP2A6* genetic variants using two-step allele-specific polymerase chain reaction (PCR) assays. The first step involved amplifying the *CYP2A6* gene containing the variation of interest (gene specific). The second step used the template from the first step and involved two parallel (allele specific) reactions, one for the wild-type allele and the other for the variant allele. For each assay, water was used as a negative control. As positive controls, DNA samples from a previously-established heterozygote variant individual, a

homozygote variant individual, and an individual without the variant allele were used (the rate of failed amplification of the control samples was less than 5%). The amplification products from the second PCR reactions were electrophoresed through agarose gel stained with ethidium bromide and visualized under UV light.

2.3.2 Assays, Primer Sets, Reaction Conditions

All DNA samples were analyzed for *CYP2A6*2,*4,*9*, and **12*. African-American subjects were further analyzed for *CYP2A6*17,*20,*23,*24,*25,*26,*27*, and **35*, and Asians were further analyzed for *CYP2A6*7, *8* and **10*. These *CYP2A6* variants are prevalent among these populations and the consequential loss or decrease in CYP2A6 enzymatic function by these variants has been characterized. *CYP2A6* genetic variants that have relatively low frequency (*CYP2A6*3, *5, *6, *11, *13, *15*), low/unclear impact on CYP2A6 function (*CYP2A6*14*), both low impact and low frequency (*CYP2A6*16, *18, *19, *21*), or have limited information regarding functional impact (*CYP2A6*22, *29, *30, *31, *32, *33, *34, *36, *37*) were not assayed. CYP2A6 primer sets specific for each genotyping assay were used and are shown in Table 2.2. CYP2A6 primers were ordered from ACGT Corporation Toronto, Ontario.

All reagents used in the PCR reactions were purchased as a kit from Fermentas (Burlington, Ontario). The kit included *Taq* polymerase enzyme, 25 mM MgCl2, 10x *Taq* PCR buffer (containing 100 mM TRIS-HCl (ph 8.8 at 25 ^{o}C), 500 mM KCL and 0.8% Nonidet P40), and 10x *Taq* PCR buffer with $(NH_4)_2SO_4$. The dNTP set (included 25 mM of each nucleotide) and 1-kb Gene Ruler DNA ladder were also purchased from Fermentas (Burlington, Ontario). The reaction conditions for the first and second amplifications of each *CYP2A6* genetic variant are shown in Table 2.3, and the PCR conditions are shown in Table 2.4. All PCR reaction mixtures for the first amplification contained 50 ng of genomic DNA, and 0.8 µl of the first

Table 2.2: Primer sets used for *CYP2A6* genotyping assays. Each assay includes a gene-specific amplication step (step 1) and an allele-specific amplification step (step 2). For each primer, the nucleotide sequence and *CYP2A6* binding location are indicated.

CYP2A6 **allele - PCR step**	**Primer name**	**Primer sequence**	**Location**
*CYP2A6*2* - 1	2A61F	5'-GCT GAA CAC AGA GCA GAT GTA CA-3'	exon 1
	2A61R	5'-GGA GGT TGA CGT GAA CTG GAA GA-3'	exon 4
*CYP2A6*2* - 2	2A62wtF	5'-CTC ATC GAC GCC CT-3'	exon 3
	2A62v1F	5'-CTC ATC GAC GCC CA-3'	exon 3
	E3R-1	5'-AAC GCA CGC GGG TTC CTC GT-3'	intron 3
*CYP2A6*4E* - 1	2Aex7F	5'-GGC CAA GAT GCC CTA CAT G-3'	exon 7
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*4E* - 2	2A6In7F1	5'-ACC CAC ATT AGA AGC TTT CTA GA-3'	intron 7
	2A7In7F1	5'-CCC CAT TAG AAG CTT TCT ACT CA-3'	intron 7
	2A6R0	5'-AGG TCA TCT AGA TTT TCT CCT ACA-3'	3' flanking
*CYP2A6*7* - 1	2A6In6F1	5'-ATT TCC TGC TCT GAG ACC-3'	intron 6
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*7* - 2	2A6*7FWT-M	5'-TCC CAG TCA CCT AAG GAA AT-3'	exon 9
	2A6*7FV-M	5'-TCC CAG TCA CCT AAG GAA AC-3'	exon 9
	2A6R0	5'-AGG TCA TCT AGA TTT TCT CCT ACA-3'	3' flanking
*CYP2A6*8* - 1	2A6In6F1	5'-ATT TCC TGC TCT GAG ACC-3'	intron 6
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*8* - 2	2A6*8WTF	5'-GCT TTG CCA CGA TCC CAC G-3'	exon 9
	2A6*8VF	5'-GCT TTG CCA CGA TCC CAC T-3'	exon 9
	2A6R0	5'-AGG TCA TCT AGA TTT TCT CCT ACA-3'	3' flanking
*CYP2A6*9* - 1	2A65Pr1F	5'-ACC TAG ACT TAA TCT TCC CGT ATA C-3'	5' flanking
	2A6In1R	5'-CCC AAG ATC CTG TCT TTC TGA T-3'	5' flanking

*CYP2A6*9* - 2	2A6-460F	5'-ATC CTC CAC AAC AGA AGA CCC CTA A-3'	5' flanking
	2A6-17RA	5'-ACG GCT GGG GTG GTT TGC CTT TA-3'	5' flanking
	2A6-17RC	5'-ACG GCT GGG GTG GTT TGC CTT TC-3'	5' flanking
*CYP2A6*10* - 1	2A6In6F1	5'-ATT TCC TGC TCT GAG ACC-3'	intron 6
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*10* - 2	2A6*7FWT-M	5'-TCC CAG TCA CCT AAG GAA AT-3'	exon 9
	2A6*7FV-M	5'-TCC CAG TCA CCT AAG GAA AC-3'	exon 9
	2A6*8Rwt-L	5'-GGA AGC TCA TGG TGT AGT TTC-3'	exon 9
	2A6*8Rv-L	5'-GGA AGC TCA TGG TGT AGT TTA-3'	exon 9
*CYP2A6*12* - 1	2AF	5'-GCA CCC CTC CTG AGG TAC CAC-3'	5' flanking
	2A6ex3R1	5'-GTC CCC TGC TCA CCG CCA-3'	exon 3
*CYP2A6*12* - 2	2A61F-L	5'-TGG CTG TGT CCC AAG CTA GGC A-3'	5' flanking
	2A71F-L	5'-TGG CTG TGT CCC AAG CTA GGT G-3'	5' flanking
	2A6ex3R2	5'-CGC TCC CCG TTG CTG AAT A-3'	exon 3
*CYP2A6*17* - 1	2A6In6F1	5'-ATT TCC TGC TCT GAG ACC-3'	intron 6
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*17* - 2	2A6*17Fwt-M	5'-GAG ATC CAA AGA TTT GGA GCC G-3'	exon 7
	2A6*17Fv-M	5'-GAG ATC CAA AGA TTT GGA GCC A-3'	exon 7
	2A6In7AS	5'-CTG AGA TTT CTG TCC CTA T-3'	intron 7
*CYP2A6*20* - 1	2A6exin3F	5'-GGC ACT GGC GGT GAG CAG-3'	exon/ intron 3
	2A6in5R	5'-GGC CTG TGT CAT CTG CCT-3'	intron 5
*CYP2A6*20* - 2	2A6in3F	5'-CTG CCT CCT GGA ATT CTG AC-3'	intron 3
	2A6ex4R2144w	5'-ACA GTG ACA GGA ACT CTT-3'	exon4
	2A6ex4R2144v	5'-ACA GTG ACA GGA ACT CTG-3'	exon 4
*CYP2A6*23* - 1	2A6exin3F	5'-GGC ACT GGC GGT GAG CAG-3'	exon/ intron 3
	2A6in5R	5'-GGC CTG TGT CAT CTG CCT-3'	intron 5

*CYP2A6*23* - 2	2A6in3F	5'-CTG CCT CCT GGA ATT CTG AC-3'	intron 3
	2A6ex42161AW	5'-GGA AGA TTC CTA GCA TCA TGC G-3'	exon4
	2A6EX42161AV	5'-GGA AGA TTC CTA GCA TCA TGC A-3'	exon 4
*CYP2A6*24* - 1	2A61F	5'-GCT GAA CAC AGA GCA GAT GTA CA-3'	exon 1
	2A61R	5'-GGA GGT TGA CGT GAA CTG GAA GA-3'	exon 4
*CYP2A6*24* - 2	2A6ex2FWT	5'-GCC ACC TTC GAC TGG G-3'	exon 2
	2A6ex2FV	5'-GCC ACC TTC GAC TGG C-3'	exon 2
	E3R-1	5'-AAC GCA CGC GGG TTC CTC GT-3'	intron 3
*CYP2A6*25* - 1	2A61F	5'-GCT GAA CAC AGA GCA GAT GTA CA-3'	exon 1
	2A61R	5'-GGA GGT TGA CGT GAA CTG GAA GA-3'	exon 4
*CYP2A6*25* - 2	2A6in2ex3FW	5'-CAC CTC CCC AGG CGT GGT AT-3'	intron 2/ exon 3
	2A6in2ex3FV	5'-CAC CTC CCC AGG CGT GGT AC-3'	intron 2/ exon3
	E3R-1	5'-AAC GCA CGC GGG TTC CTC GT-3'	intron 3
*CYP2A6*26* - 1	2A61F	5'-GCT GAA CAC AGA GCA GAT GTA CA-3'	exon 1
	2A61R	5'-GGA GGT TGA CGT GAA CTG GAA GA-3'	exon 4
*CYP2A6*26* - 2	2A6ex2FWT	5'-GCC ACC TTC GAC TGG G-3'	exon 2
	2A6ex3R1711w	5'-GCA GGG TGG CGA TGG A-3'	exon 3
	2A6ex3R1711v	5'-GCA GGG TGG CGA TGG C-3'	exon 3
*CYP2A6*27* - 1	2A6exin3F	5'-GGC ACT GGC GGT GAG CAG-3'	exon3/ intron3
	2A6in5R	5'-GGC CTG TGT CAT CTG CCT-3'	intron 5
*CYP2A6*27* - 2	2A6in3F	5'-CTG CCT CCT GGA ATT CTG AC-3'	intron 3
	2A6*4171W-M	5'-GGA AGA TTC CTA GCA TCC TG-3'	exon 4
	2A6*4171V-M	5'-GGA AGA TTC CTA GCA TCC TT-3'	exon 4
*CYP2A6*35* - 1	2A6In6F1	5'-ATT TCC TGC TCT GAG ACC-3'	intron 6
	2A6R6	5'-TAA TTG GGT TGT TTT CTA TTG AGT-3'	3' flanking
*CYP2A6*35* - 2	2A6in8ex9F6458W	5'-TCC TCA GGA AAG CGG A-3'	intron8/exon9
	2A6in8ex9F6458V	5'-TCC TCA GGA AAG CGG T-3'	intron8/exon9
	2A6R0	5'-AGG TCA TCT AGA TTT TCT CCT ACA-3'	3' flanking

Table 2.3: Reaction conditions for step 1 and 2 of each *CYP2A6* genotyping assay used in this study.

CYP2A6 allele	PCR step	Reaction Conditions					
		DNA	Primers (mM)	PCR buffer	MgCl2 (mM)	dNTPs (mM)	Taq polymerase (Units)
*CYP2A6*2*	1	50 ng	0.25	1X	2	0.2	0.75
	2	0.8 µL	0.25	1X	1.25	0.1	0.25
*CYP2A6*4E*	1	50 ng	0.125	1X	1.7	0.2	1.25
	2	0.8 µL	0.25	1X	1.5	0.1	0.3
*CYP2A6*7*	1	50 ng	0.25	1X	1.5	0.2	1
	2	0.8 µL	0.25	1X	1.1	0.1	0.3
*CYP2A6*8*	1	50 ng	0.25	1X	1.5	0.2	1
	2	0.8 µL	0.25	1X $(NH_4)_2SO_4$	1	0.1	0.3
*CYP2A6*9*	1	50 ng	0.125	1X	1.3	0.2	0.75
	2	0.8 µL	0.125	1X $(NH_4)_2SO_4$	1.1	0.1	0.4
*CYP2A6*10*	1	50 ng	0.25	1X	1.5	0.2	1
	2	0.8 µL	0.2	1X	1.2	0.1	0.3
*CYP2A6*12*	1	50 ng	0.25	1X	1.5	0.2	0.6
	2	0.8 µL	0.125	1X	1.5	0.1	0.5
*CYP2A6*17*	1	50 ng	0.25	1X	1.5	0.2	1
	2	0.8 µL	0.125	1X $(NH_4)_2SO_4$	1	0.1	0.5
*CYP2A6*20*	1	50 ng	0.125	1X	1.5	0.2	1.25
	2	0.8 µL	0.25	1X	1.5	0.1	0.3
*CYP2A6*23*	1	50 ng	0.125	1X	1.5	0.2	1.25
	2	0.8 µL	0.15	1X $(NH_4)_2SO_4$	1	0.1	0.4
*CYP2A6*24*	1	50 ng	0.25	1X	2	0.2	0.75
	2	0.8 µL	0.15	1X	1.25	0.1	0.3
*CYP2A6*25*	1	50 ng	0.25	1X	2	0.2	0.75
	2	0.8 µL	0.25	1X	0.75	0.1	0.3
*CYP2A6*26*	1	50 ng	0.25	1X	2	0.2	0.75
	2	0.8 µL	0.125	1X	1.2	0.1	0.4
*CYP2A6*27*	1	50 ng	0.125	1X	1.5	0.2	1.25
	2	0.8 µL	0.25	1X	1.3	0.1	0.4
*CYP2A6*35*	1	50 ng	0.25	1X	1.5	0.2	1
	2	0.8 µL	0.25	1X	1.25	0.1	0.5

Table 2.4: PCR conditions for step 1 and 2 of each *CYP2A6* genotyping assay used in this study.

CYP2A6 **allele**	**PCR step**	**Initial denaturation**		**Denaturation**		**Annealing**		**Extension**		**Final extension**		**Cycles**
		Temp (°C)	**Time (min)**	**Temp (°C)**	**Time (min)**	**Temp (°C)**	**Time (min)**	**Temp (°C)**	**Time (min)**	**Temp (°C)**	**Time (min)**	
*CYP2A6*2*	1	95	1:00	95	0:15	60	0:20	72	3:00	72	7:00	36
	2	95	1:00	95	0:15	50	0:20	72	0:45			22
*CYP2A6*4E*	1	95	1:00	95	0:15	50	0:40	72	2:00	72	7:00	40
	2	95	1:00	95	0:15	50	0:20	72	2:00			20
*CYP2A6*7*	1	95	1:00	95	0:15	50	0:30	72	3:00	72	7:00	40
	2	95	1:00	95	0:15	59	0:20	72	1:00			30
*CYP2A6*8*	1	95	1:00	95	0:15	50	0:30	72	3:00	72	7:00	40
	2	95	1:00	95	0:15	57	0:20	72	1:00			20
*CYP2A6*9*	1	95	1:00	95	0:20	55	0:30	72	2:00	72	7:00	30-40
	2	94	1:00	94	0:20	66	0:40	72	1:00			18-20
*CYP2A6*10*	1	95	1:00	95	0:15	50	0:30	72	3:00	72	7:00	40
	2	95	1:00	95	0:15	58	0:30	72	0:30			22
*CYP2A6*12*	1	95	1:00	95	0:15	58	0:30	72	2:00	72	7:00	35
	2	95	1:00	95	0:15	63	0:20	72	1:00			20
*CYP2A6*17*	1	95	1:00	95	0:15	50	0:30	72	3:00	72	7:00	40
	2	95	1:00	95	0:15	58	0:30	72	1:00			20
*CYP2A6*20*	1	95	1:00	95	0:15	58	0:30	72	2:00	72	7:00	30
	2	95	1:00	95	0:15	56	0:20	72	0:30			18
*CYP2A6*23*	1	95	1:00	95	0:15	58	0:30	72	2:00	72	7:00	30
	2	95	1:00	95	0:15	60	0:10	72	0:30			16-20
*CYP2A6*24*	1	95	1:00	95	0:15	60	0:20	72	3:00	72	7:00	36
	2	95	1:00	95	0:15	58	0:20	72	1:30			24
*CYP2A6*25*	1	95	1:00	95	0:15	60	0:20	72	3:00	72	7:00	36
	2	95	1:00	95	0:15	65	0:20	72	0:45			18
*CYP2A6*26*	1	95	1:00	95	0:15	60	0:20	72	3:00	72	7:00	36
	2	95	1:00	95	0:15	59	0:20	72	1:00			18
*CYP2A6*27*	1	95	1:00	95	0:15	58	0:30	72	2:00	72	7:00	30
	2	95	1:00	95	0:15	56	0:20	72	0:40			18
*CYP2A6*35*	1	95	1:00	95	0:15	50	0:30	72	3:00	72	7:00	40
	2	95	1:00	95	0:15	55	0:40	72	1:00			20

amplification product was used for the second amplification. All PCR reaction mixtures used 1x Taq PCR buffer with KCl, except for the 2nd PCR amplification steps of the *CYP2A6*8, *9, *17*, and **23* assays, which used 1x Taq PCR buffer with $(NH_4)_2SO_4$. The total volume for all reactions was 25 µl. PCR amplifications were carried out on a PTC-200 Peltier Thermal Cycler (BioRad, Toronto, ON, Canada). For all PCR amplifications, the following steps were followed: 1) initial denaturation; 2) denaturation; 3) annealing; 4) extension; 5) repeat of steps 2-4 (denaturation, annealing, extension) for a specified number of cycles; and 6) final extension (only for the 1st amplification reaction).

2.3.3 Gel Electrophoresis and Visualization

The total volume of PCR product from each second amplification was mixed with either 2.5 µl of 0.25% Bromophenol Blue or Xylene Cyanol FF loading dye (both dyes contain 30% glycerol), and 20 µl from each reaction was loaded onto a selected percentage agarose gel (Table 2.5). The selection of loading dye and agarose gel percentage depended on the product size of second PCR amplification. Agarose was purchased from ONBIO Inc. (Richmond Hill, ON), and 10x TEA buffer and ethidium bromide (10mg/mL solution) were purchased from Sigma, Aldrich. Gels were made in a total volume of 350 mL 1x TAE buffer (0.4M Tris base, 0.02 M acetic acid, 0.001 M EDTA) and stained with ethidium bromide (60 µg ethidium bromide per 100 mL agarose gel). Once loaded into the gel, samples were electrophoresed at 90-100V for 30-60 minutes and visualized using the AlphaDigiDoc real time imaging system (Alpha Innotech, Fisher Scientific). The presence/absence of bands on the gel indicated whether an individual did or did not have the *CYP2A6* variant allele being assayed. A total *CYP2A6* genotype for each individual was determined once all *CYP2A6* assays were complete.

Table 2.5: Loading dye and gel composition used for visualization of PCR products from each *CYP2A6* genotyping assay.

Assay	Product size (base pairs)	Loading dye	Gel composition (% agarose)
*CYP2A6*2*	97	Xylene	3
*CYP2A6*4E*	2584	Bromophenol Blue	1.2
*CYP2A6*7*	1244	Bromophenol Blue	1.2
*CYP2A6*8*	1201	Bromophenol Blue	1.2
*CYP2A6*9*	408	Xylene	1.2
*CYP2A6*10*	82	Xylene	3
*CYP2A6*12*	1831	Bromophenol Blue	1.2
*CYP2A6*17*	339	Xylene	1.2
*CYP2A6*20*	227	Xylene	1.5
*CYP2A6*23*	212	Xylene	1.5
*CYP2A6*24*	1302	Bromophenol Blue	1.2
*CYP2A6*25*	228	Xylene	1.5
*CYP2A6*26*	1100	Bromophenol Blue	1.2
*CYP2A6*27*	212	Xylene	1.5
*CYP2A6*35*	1340	Bromophenol Blue	1.2

2.3.4 *CYP2A6* Genotype Grouping

According to *CYP2A6* genotype, all subjects were catagorized into the SM, IM or NM group (shown in Table 1.4), as done previously (Schoedel *et al.* 2004; Malaiyandi *et al.* 2005; Ho *et al.* 2009). Smokers with at least one of *CYP2A6*2*, **4*, *7, *10, **17, *20, *23, *24, *25, *26, *27* or **35* alleles, or two of *CYP2A6*9* or **12* alleles, were grouped as SMs. Smokers with just one *CYP2A6*9* or **12* allele were grouped as IMs. All other subjects (i.e. those without the above *CYP2A6* variant alleles) were grouped as NMs.

2.4 Statistical Analysis

To assess whether *CYP2A6* genotype frequencies were in Hardy-Weinberg equilibrium and to compare *CYP2A6* allele frequencies between this study and those reported in the literature, Chi-tests were used. Categorical and continuous population characteristics were compared across treatment groups and genotype groups using Chi-tests or ANOVA followed by Bonferroni multiple comparisons test (if ANOVA $p<0.05$). The 3HC/COT ratios obtained for this study population were not normally distributed and therefore were log-transformed for statistical analyses. 3HC/COT for all genotypes were compared to a reference group using unpaired t-tests or ANOVA followed by Bonferroni multiple comparisons test (if ANOVA $p<0.05$). The three genotype groups were compared by 3HC/COT, CPD, FTND, and plasma measures using ANOVA followed by Bonferroni multiple comparisons test (if ANOVA $p<0.05$). To compare abstinence between genotype and 3HC/COT groups, Chi-tests were used. All statistical analyses were performed using GraphPad Prism.

3. RESULTS

3.1 *CYP2A6* Allele and Genotype Group Frequencies

In this study 570 DNA samples (482 Caucasians, 84 Blacks, 4 Asians) were received and 557 were successfully genotyped (471 Caucasians, 82 Blacks, 4 Asians) for *CYP2A6*2, *4E, *9*, and **12*, plus numerous additional alleles found at a higher frequency in the Black and Asian populations. Examples of genotyping results for some of the assays are shown in Figure 3.1. The observed allele frequencies in this study are shown in Table 3.1. *CYP2A6*2,*4E, *9* and **12* allele frequencies were calculated among the entire study population (n=557), and separately among Caucasians (n=471) and Blacks (n=82); *CYP2A6*17,*20,*23, *24, *25, *26, *27*, and **35* allele frequencies were calculated only among the Black population (n=82); and *CYP2A6*7, *8* and **10* allele frequencies were calculated only among the Asian subjects (n=4). Chi-tests were used to compare observed allele frequencies with allele frequencies previously reported in smoking populations of similar ethnicity. Observed allele frequencies were all similar to previously reported frequencies in smoking populations, except for *CYP2A6*4*, which had a significantly higher frequency in our current Caucasian population compared to previous findings by Malaiyandi *et al*. (2006) (1.1% vs 0.13%) (X^2=5.78, df=1, p=0.02). One possible reason for the increased frequency in our study is that we used a *CYP2A6*4* assay that detects variants *CYP2A6*4A*, *4*D* and *4*E*, while Malaiyandi *et al.* (2006) used an assay that only detected *CYP2A6*4A* and *4*D*. The increased detection of *CYP2A6*4* in our study was desired because the relatively low frequency reported by Malaiyandi *et al.* (2006) reduced their power to detect an effect of *CYP2A6* on quitting rates with nicotine patch and spray.

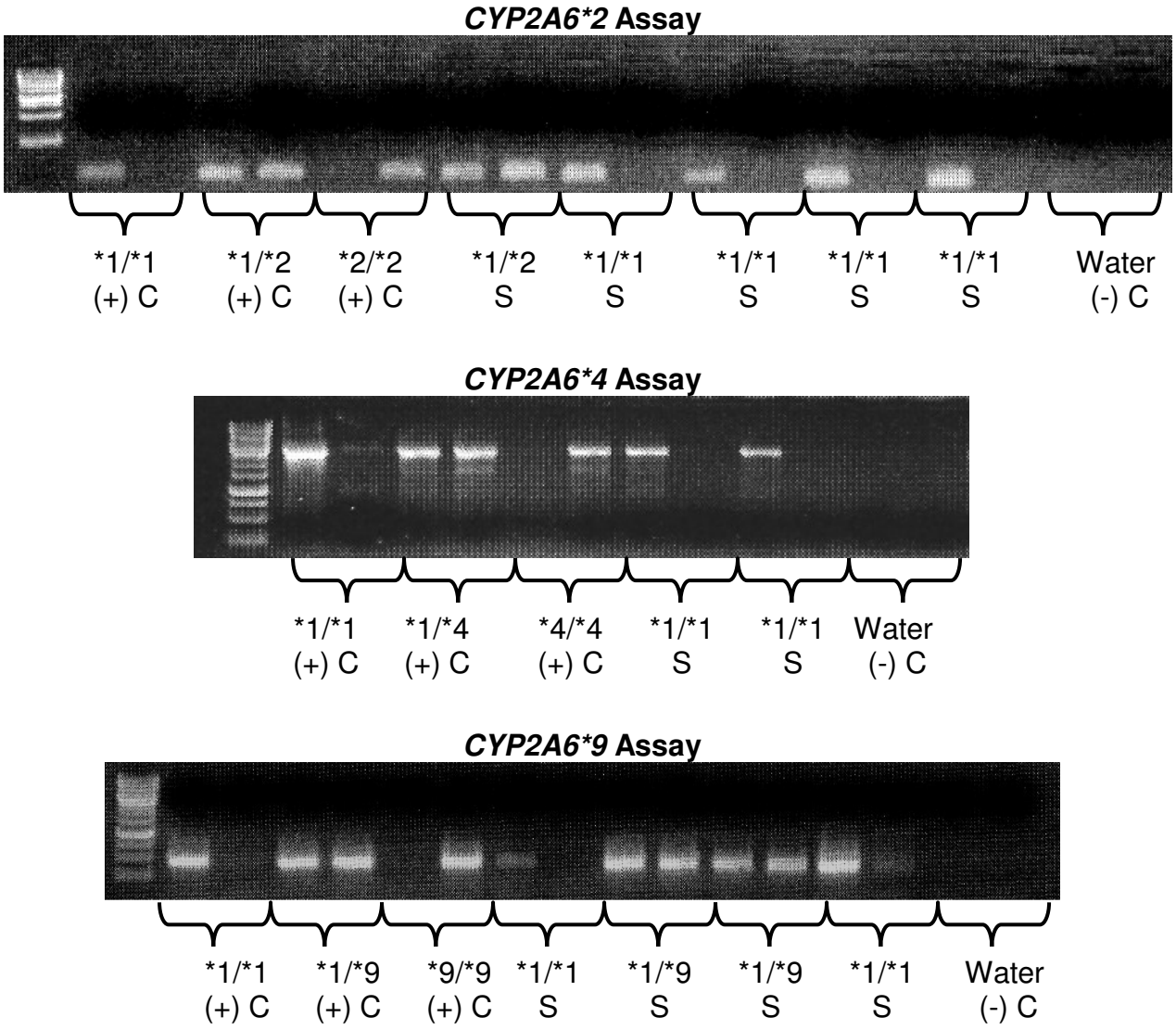


Figure 3.1: Example photographs of 2nd amplification results for a *CYP2A6*2, CYP2A6*4* and *CYP2A6*9* genotyping assay. For each sample (S), the products from the reaction containing the primer set specific for the wildtype allele (*CYP2A6*1*) and for the variant allele were loaded sequentially (i.e. two lanes per sample). Amplification only in the 1st lane of each pair indicated that the individual did not have the variant allele. If amplification was present in both lanes, this indicated that the individual had at least one non-variant allele and variant allele. Amplification only in the 2nd lane of each pair indicated that the individual was homozygous for the *CYP2A6* variant allele. A 1kb ladder (Fermentas) was loaded for each gel. Water was used as the negative control [(-) C] for all assays. DNA samples from a previously-established heterozygote variant individual, a homozygote variant individual, and an individual without the variant allele were used as positive controls [(+) C] for each reaction assay and loaded onto the gel. The rate of failed amplification of the control samples was less than 5%.
(+) C = positive control; (-) C = negative control; S = sample

Table 3.1: Observed *CYP2A6* allele frequencies amongst all participants (n=557) successfully genotyped for *CYP2A6* variants. The allele frequencies in our present study and in previous studies were reported and compared by Chi-test.

Allele	Present study		Previous studies (smoking populations)			X^2 test p-value [d]
	Total alleles [b]	Observed frequency (n)	Total alleles	Observed frequency	Reference	
*CYP2A6*2*	1114	2.9% (32)	-	-	-	-
	942 (Caucasians)	3.3% (31)	788	2.0% (16)	(Malaiyandi *et al.* 2006)	0.11
	164 (Blacks)	0.6% (1)	1236	0.9% (11)	(Ho *et al.* 2009)	0.71
*CYP2A6*4* [a]	1114	1.1% (12)	-	-	-	-
	942 (Caucasians)	1.1% (10)	772	0.13% (1)	(Malaiyandi *et al.* 2006)	**0.02**
	164 (Blacks)	1.2%(2)	1236	1.9% (23)	(Ho *et al.* 2009)	0.56
*CYP2A6*7*	8 (Asians)	0% (0)	1846	9% (166) [c]	(Mwenifumbo *et al.* 2005)	0.37
*CYP2A6*8*	8 (Asians)	0% (0)	1846	0.05% (1) [c]	(Mwenifumbo *et al.* 2005)	0.95
*CYP2A6*9*	1114	6.8% (76)	-	-	-	-
	942 (Caucasians)	6.7% (64)	766	8.0% (61)	(Malaiyandi *et al.* 2006)	0.36
	164 (Blacks)	7.3% (12)	1236	9.6% (119)	(Ho *et al.* 2009)	0.34
*CYP2A6*10*	8 (Asians)	0% (0)	1846	4% (75) [c]	(Mwenifumbo *et al.* 2005)	0.52
*CYP2A6*12*	1114	1.0% (11)	-	-	-	-
	942 (Caucasians)	1.2% (11)	766	2.1% (16)	(Malaiyandi *et al.* 2006)	0.13
	164 (Blacks)	0% (0)	1236	0.4% (5)	(Ho *et al.* 2009)	0.41
*CYP2A6*17*	164 (Blacks)	11.0% (18)	1236	8.0% (99)	(Ho *et al.* 2009)	0.20
*CYP2A6*20*	164 (Blacks)	0.6% (1)	1236	1.5% (19)	(Ho *et al.* 2009)	0.35
*CYP2A6*23*	164 (Blacks)	1.8% (3)	1236	1.1% (14)	(Ho *et al.* 2009)	0.44
*CYP2A6*24*	164 (Blacks)	0% (0)	1236	0.7% (9)	(Ho *et al.* 2009)	0.27
*CYP2A6*25*	164 (Blacks)	0% (0)	1236	0.9% (11)	(Ho *et al.* 2009)	0.23
*CYP2A6*26*	164 (Blacks)	0.6% (1)	1236	0.7% (9)	(Ho *et al.* 2009)	0.87
*CYP2A6*27*	164 (Blacks)	0% (0)	1236	0.7% (9)	(Ho *et al.* 2009)	0.27
*CYP2A6*35*	164 (Blacks)	1.8% (3)	1236	2.9% (36)	(Ho *et al.* 2009)	0.43

[a] The *CYP2A6*4E* assay was used in the present study, which detected variants **4A*, **4D* and **4E*. The previous study in Caucasians by Malaiyandi *et al*. (2006) used a *CYP2A6*4A&D* assay, which detected **4A* and **4D*.
[b] The *CYP2A6*2,*4, *9* and **12* allele frequencies were calculated among the total population (n=557), Caucasians (n=471), and Blacks (n=82). *CYP2A6*7, *8* and **10* allele frequencies were calculated only among Asians (n=4). *CYP2A6*17,*20,*23, *24, *25, *26, *27*, and **35* allele frequencies were calculated only among Blacks (n=82).
[c] These were pooled observations from a population of Chinese-Canadians and -Americans, Taiwanese, Korean-Americans and Japanese-Canadians.
[d] The allele frequencies obtained in our Caucasian and Black populations were compared to previously reported frequencies in similar ethnic groups.

Table 3.2: Frequency of *CYP2A6* genotypes and their associated baseline mean 3HC/COT ± SD among all participants (n=557). The baseline mean 3HC/COT for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group.

Allele	Genotype	Observed frequency (n)	Baseline mean 3HC/COT [a]	SD	% Mean	p-value[b]
Reference	*1/*1	413	0.42	0.19	100%	-
*CYP2A6*2*	*1/*2	22	0.26	0.14	62%	<0.0001
	*2/*2	3	0.11	0.08	26%	
*CYP2A6*4*	*1/*4	9	0.31	0.17	75%	0.02
*CYP2A6*9*	*1/*9	63	0.29	0.21	69%	<0.0001
	*9/*9	4	0.15	0.07	35%	
*CYP2A6*12*	*1/*12	11	0.21	0.09	50%	<0.0001
*CYP2A6*17*	*1/*17	13	0.29	0.13	69%	<0.0001
	*17/*17	2	0.08	0.06	18%	
*CYP2A6*20*	*1/*20	1	0.19	-	46%	-
*CYP2A6*23*	*1/*23	1	0.14	-	34%	0.25
	*23/*23	1	0.51	-	122%	
*CYP2A6*35*	*1/*35	2	0.40	0.15	95%	1.00
Two or more different variant alleles	*2/*9	1	0.65	-	156%	<0.0001
	*4/*9	1	0.04	-	10%	
	*4/*12	1	0.02	-	5%	
	*4/*26	1	0.02	-	5%	
	*4/*35	1	0.07	-	17%	
	*9/*17	1	0.12	-	29%	
	*2/*2/*9	1	0.32	-	77%	

[a] Baseline mean 3HC/COT ratios reported in the table were pre-log transformed.
[b] For statistical analysis, the ratios were log-transformed to obtain normal distribution. ANOVA or student's t-test were used to compare the log-transformed mean 3HC/COT ratio from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with the *CYP2A6*23* allele were grouped for analysis, as were individuals with two or more different variant alleles.

Individual genotype frequencies among all participants (n=557) are shown in Table 3.2. Genotype frequencies were calculated separately among Caucasians (n=471) and Blacks (n=82), and are shown in Table 3.3 and Table 3.4, respectively. The frequencies of each of the genotypes did not deviate significantly from Hardy-Weinberg equilibrium (X^2 $p>0.20$).

The observed *CYP2A6* genotype group frequencies among all subjects was 74.7% NM, 13.4% IM and 11.8% SM (Table 3.5). When the study group was divided into Caucasians and Blacks, there were significant differences in the proportion of metabolizer groups between these two populations ($X^2=35.06$, df=2, $p<0.0001$). Caucasians were composed of 79.3% NM, 14.3% IM and 8.7% SM, while the Black group was 57.8% NM, 10.8% IM and 31.3% SM. Genotype group frequencies were similar to previously reported frequencies in Caucasian ($X^2=3.12$, df=2, $p=0.21$) and Black smoking populations ($X^2=2.09$, df=2, $p=0.35$).

3.2 Participant Characteristics by Treatment Group and *CYP2A6* Genotype Group

Baseline participant characteristics for all subjects (n=568) and by treatment group (n=286 for standard treatment, n=282 for extended treatment) are shown in Table 3.6. The student's t-test was used to compare age, BMI, FTND scores, CPD, age of smoking initiation and baseline nicotine, COT and 3HC between the standard and extended treatment groups. The Chi-test was used to compare gender and proportion of metabolizer groups between the treatment groups. There were no significant differences in any baseline characteristics between treatment groups. From here on in, an additional combined group of IMs and SMs (termed reduced metabolizers, RM) was included in our analyses, given that RMs were included in our analysis of abstinence rates (**section 3.6**).[1] Baseline participant characteristics for all participants (n=557) in each *CYP2A6* genotype group are shown in Table 3.7, and only among Caucasians (n=468) in

[1] The RM group was a pooled group of SMs and IMs, plus 5 individuals that could be either IM or SM due to incomplete genotyping (yet we still knew that they had at least one *CYP2A6*9* or *CYP2A6*12* variant).

Table 3.3: Frequency of *CYP2A6* genotypes and their associated baseline mean 3HC/COT ± SD among Caucasians (n=471). The baseline mean 3HC/COT for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group.

Allele	Genotype	Observed frequency (n)	Baseline mean 3HC/COT [a]	SD	% Mean	p-value[b]
Reference	*1/*1	362	0.42	0.19	100%	-
*CYP2A6*2*	*1/*2	21	0.24	0.10	57%	<0.0001
	*2/*2	3	0.12	0.08	29%	
*CYP2A6*4*	*1/*4	9	0.31	0.17	74%	0.01
*CYP2A6*9*	*1/*9	53	0.29	0.12	69%	<0.0001
	*9/*9	3	0.15	0.08	36%	
*CYP2A6*12*	*1/*12	11	0.21	0.09	50%	<0.0001
Two or more different variant alleles	*2/*9	1	0.65	-	155%	<0.0001
	*4/*9	1	0.04	-	10%	
	*4/*12	1	0.02	-	5%	
	*2/*2/*9	1	0.32	-	76%	

[a] Baseline mean 3HC/COT ratios reported in the table were pre-log transformed.
[b] For statistical analysis, the ratios were log-transformed to obtain normal distribution. ANOVA or student's t-test were used to compare the log-transformed mean 3HC/COT ratio from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with two or more different variant alleles were grouped for analysis.

Table 3.4: Frequency of *CYP2A6* genotypes and their associated baseline mean 3HC/COT ± SD among Blacks (n=82). The baseline mean 3HC/COT for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group.

Allele	Genotype	Observed frequency (n)	Baseline mean 3HC/COT [a]	SD	% Mean	p-value[b]
Reference	*1/*1	47	0.38	0.20	100%	-
*CYP2A6*2*	*1/*2	1	0.69	-	182%	-
*CYP2A6*9*	*1/*9	9	0.28	0.12	74%	0.04
	*9/*9	1	0.13	-	34%	
*CYP2A6*17*	*1/*17	13	0.29	0.13	76%	<0.0001
	*17/*17	2	0.08	0.06	21%	
*CYP2A6*20*	*1/*20	1	0.19	-	50%	-
*CYP2A6*23*	*1/*23	1	0.14	-	37%	0.50
	*23/*23	1	0.51	-	134%	
*CYP2A6*35*	*1/*35	2	0.40	0.15	105%	0.73
Two or more different variant alleles	*4/*26	1	0.02	-	5%	<0.0001
	*4/*35	1	0.07	-	18%	
	*9/*17	1	0.12	-	32%	

[a] Baseline mean 3HC/COT ratios reported in the table were pre-log transformed.
[b] For statistical analysis, the ratios were log-transformed to obtain normal distribution. ANOVA or student's t-test were used to compare the log-transformed mean 3HC/COT ratio from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with a *CYP2A6*9* or **23* allele were grouped for analysis, as were individuals with two or more different variant alleles.

Table 3.5: Frequency of *CYP2A6* genotype groups among all partcipants (n=558), and among only Caucasian (n=471) and Black (n=83) participants. The genotype group frequencies in our present study and in previous studies were reported and compared by Chi-test.

	Present study				Previous studies (smoking populations)				X^2 test p-value
Study participants	**Normal (n)**	**Intermediate (n)**	**Slow (n)**	**X^2 test p-value**	**Normal (n)**	**Intermediate (n)**	**Slow (n)**	**Reference**	
All	74.7% (417)	13.4% (75)	11.8% (66)	-	-	-	-	-	-
Caucasians[a]	79.3% (365)	14.3% (66)	8.7% (40)	<0.0001[c]	78.4% (309)	16.0% (63)	5.6% (22)	Malaiyandi *et al.* (2006)	0.21[d]
Blacks[b]	57.8% (48)	10.8% (9)	31.3% (26)		49.7% (246)	14.9% (74)	35.4% (175)	Ho *et al.* (2009)	0.35[e]

[a] Caucasians were grouped based on the *CYP2A6*2, *4, *9* and **12* alleles.
[b] Blacks were grouped based on the *CYP2A6*2, *4, *9*, **12, *17, *20, *23, *24, *25, *26, *27*, and **35* alleles.
[c] P-value reflects a comparison of the proportion of genotype groups among Caucasians to the proportion of genotype groups among Blacks in this study.
[d] P-value reflects a comparison of the proportion of genotype groups among Caucasians in this study to the proportion of genotype groups among Caucasians in Malaiyandi *et al.* (2006).
[e] P-value reflects a comparison of the proportion of genotype groups among Blacks in this study to the proportion of genotype groups among Blacks in Ho *et al.* (2009).

Table 3.6: Baseline characteristics reported for the total study population and by treatment group.

	All (n=568)		Standard treatment (n=286)		Extended treatment (n=282)		p-value
Characteristic	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**t-test[a]**
Age	44.8	10.3	44.9	10.4	44.8	10.2	0.94
BMI	28.9	5.9	28.8	5.8	28.9	5.9	0.97
FTND	5.3	2.1	5.3	2.1	5.2	2.2	0.84
CPD	21.2	9.2	21.3	9.0	21.1	9.5	0.92
Age of initiation	15.7	4.2	15.7	4.3	15.8	4.0	0.99
Baseline nicotine (ng/mL)	17.8	9.2	17.8	8.5	17.8	9.9	0.99
Baseline COT (ng/mL)	269	117	273	110	266	124	0.77
Baseline 3HC (ng/mL)	99	61	103	60	95	61	0.52
							X^2 test[b]
% female	44.7%		45.1%		44.3%		0.85
% *CYP2A6* NM	74.8%		77.7%		71.9%		0.28
% *CYP2A6* IM	13.4%		12.2%		14.6%		
% *CYP2A6* SM	11.8%		10.1%		13.5%		
% *CYP2A6* RM	25.9%		23.1%		28.6%		

[a] P-value reflects a comparison (by student's t-test) of the baseline characteristic between standard and extended treatment groups.
[b] P-value reflects a comparison (by Chi-test) of gender proportion or genotype group proportion between standard and extended treatment groups.

Table 3.7: Baseline characteristics for the total study population (n=557) reported by *CYP2A6* genotype group.

	NM (n=413)		IM (n=74)		SM (n=65)		RM (n=144)[a]		p-value	
Characteristic	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**ANOVA[b]**	**Post-hoc**
Age	44.9	10.5	43.9	10.1	45.1	9.4	44.8	9.8	0.88	
BMI	28.8	5.8	28.6	5.7	30.1	6.5	29.2	6.0	0.36	
FTND	5.3	2.1	5.4	2.0	4.8	2.1	5.1	2.1	0.15	
CPD	21.7	9.4	21.6	9.2	17.9	7.8	20.0	8.8	0.01	<0.01 (NM vs SM)
Age of initiation	16.0	4.4	14.8	3.3	15.2	3.3	15.0	3.3	0.01	<0.05 (NM vs RM)
Baseline nicotine (ng/mL)	17.4	9.0	19.5	10.3	19.3	9.4	19.4	9.7	0.06	
Baseline COT (ng/mL)	268	118	288	115	259	112	276	115	0.44	
Baseline 3HC (ng/mL)	109	62	80	48	66	50	74	49	<0.0001	<0.001 (NM vs SM) <0.001 (NM vs IM) <0.001 (NM vs RM)
Baseline 3HC/COT[d]	0.42	0.20	0.28	0.12	0.25	0.16	0.26	0.14	<0.0001	<0.001 (NM vs SM) <0.001 (NM vs IM) <0.001 (NM vs RM) <0.05 (IM vs SM)
									X^2 test[c]	
% female	45.0%		51.4%		49.2%		52.1%		0.43	

[a] RM genotype group was a pooled group of SMs and IMs, plus 5 individuals that were either IM or SM (unknown due to incomplete genotyping).
[b] Baseline characteristics by genotype group were compared by ANOVA followed by Bonferroni's multiple comparison test if ANOVA $p<0.05$.
[c] Gender proportion by genotype group was compared by Chi-test.
[d] Baseline 3HC/COT ratios reported in the table are pre-log transformed. For statistical analysis, ratios were log-transformed to obtain normal distribution.

Table 3.8. ANOVA followed by the Bonferroni's multiple comparison test was used to compare age, BMI, FTND scores, CPD, age of smoking initiation and baseline nicotine, COT and 3HC between the *CYP2A6* metabolizer groups. The Chi-test was used to compare gender. Among all participants, there were significant differences in CPD (ANOVA p=0.01), age of smoking initiation (ANOVA p=0.01), baseline 3HC (ANOVA p<0.0001) and 3HC/COT (ANOVA p<0.0001) between genotype groups. Similar differences were found among Caucasians, except that in Caucasians there were no differences in CPD between genotype groups (ANOVA p=0.15). Differences in CPD, age of initiation and baseline 3HC will be discussed further in **section 3.4.2**, and differences in baseline 3HC/COT will be discussed further in **section 3.3**.

3.3 Association of CYP2A6 activity with *CYP2A6* Genotype Group

The pre-log transformed 3HC/COT quartile means, medians and ranges for all subjects (N=568; n=142 per quartile) were as follows: Q1) 0.18, 0.20 (<0.26); Q2) 0.30, 0.30 (0.26-0.35); Q3) 0.40, 0.39 (0.35-0.47); and Q4) 0.63, 0.56 (>0.47) (Table 3.9A). For Caucasians, the pre-log transformed 3HC/COT quartile means, medians and ranges were as follows (N=478; n=119/120 per quartile): Q1) 0.20, 0.21 (<0.27); Q2) 0.31, 0.31 (0.27-0.36); Q3) 0.41, 0.41 (0.36-0.48); and Q4) 0.63, 0.57 (>0.48) (Table 3.9B).

The distribution of 3HC/COT ratios for each *CYP2A6* genotype amongst all participants (n=556) is shown in Figure 3.2. The mean 3HC/COT ± SD for each *CYP2A6* genotype for all subjects is summarized in Table 3.2, as well as separately for Caucasians in Table 3.3 and Blacks Table 3.4. The 3HC/COT ratios were not normally distributed and were log transformed for statistical analysis. ANOVA or student's t-test was used to compare the mean 3HC/COT for each variant genotype to the *CYP2A6*1/*1* group. Among the total population and among Caucasians,

Table 3.8: Baseline characteristics of Caucasians participants (n=468) reported by *CYP2A6* genotype group.

	NM (n=363)		IM (n=65)		SM (n=40)		RM (n=110) [a]		p-value	
Characteristic	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**ANOVA[b]**	**Post-hoc**
Age	44.7	10.6	43.2	9.9	45.1	9.6	44.4	9.9	0.72	
BMI	28.6	5.7	28.5	5.2	29.0	6.1	28.7	5.5	0.97	
FTND	5.3	2.2	5.4	2.1	4.6	2.1	5.1	2.1	0.17	
CPD	22.4	9.3	21.0	7.2	20.2	8.8	20.8	7.9	0.15	
Age of initiation	16.0	4.4	14.6	3.0	15.0	3.4	14.8	3.2	0.01	<0.05 (NM vs RM)
Baseline nicotine (ng/mL)	17.6	8.2	19.1	10.2	21.3	9.2	19.9	9.6	0.02	>0.05
Baseline COT (ng/mL)	267	115	276	114	267	112	274	114	0.91	
Baseline 3HC (ng/mL)	110	60	76	46	66	49	73	47	<0.0001	<0.001 (NM vs SM) <0.001 (NM vs IM) <0.001 (NM vs RM)
Baseline 3HC/COT [c]	0.42	0.20	0.28	0.12	0.25	0.16	0.26	0.13	<0.0001	<0.001 (NM vs SM) <0.001 (NM vs IM) <0.001 (NM vs RM) <0.05 (IM vs SM)
									X^2 test [c]	
% female	42.4%		38.5%		42.5%		39.1%		0.89	

[a] RM genotype group was a pooled group of SMs and IMs, plus 5 individuals that were either IM or SM (unknown due to incomplete genotyping).
[b] Baseline characteristics by genotype group were compared by ANOVA followed by Bonferroni's multiple comparison test if ANOVA $p<0.05$.
[c] Gender proportion by genotype group was compared by Chi-test.
[d] Baseline 3HC/COT ratios reported in the table are pre-log transformed. For statistical analysis, ratios were log-transformed to obtain normal distribution.

Table 3.9A: 3HC/COT quartiles for the total study population (n=568). For each quartile, the sample size, mean 3HC/COT, median 3HC/COT and upper and lower limits of 3HC/COT are shown.

Quartile	Sample size	Mean 3HC/COT	Median 3HC/COT	Range
1	142	0.18	0.20	<0.26
2	142	0.30	0.30	0.26 - 0.35
3	142	0.40	0.39	0.35 - 0.47
4	142	0.63	0.56	>0.47

Table 3.9B: 3HC/COT quartiles for the Caucasian population (n=478). For each quartile, the sample size, mean 3HC/COT, median 3HC/COT and upper and lower limits of 3HC/COT are shown.

Quartile	Sample size	Mean 3HC/COT	Median 3HC/COT	Range
1	119	0.20	0.21	<0.27
2	120	0.31	0.31	0.27 - 0.36
3	120	0.41	0.41	0.36 - 0.48
4	119	0.63	0.57	>0.48

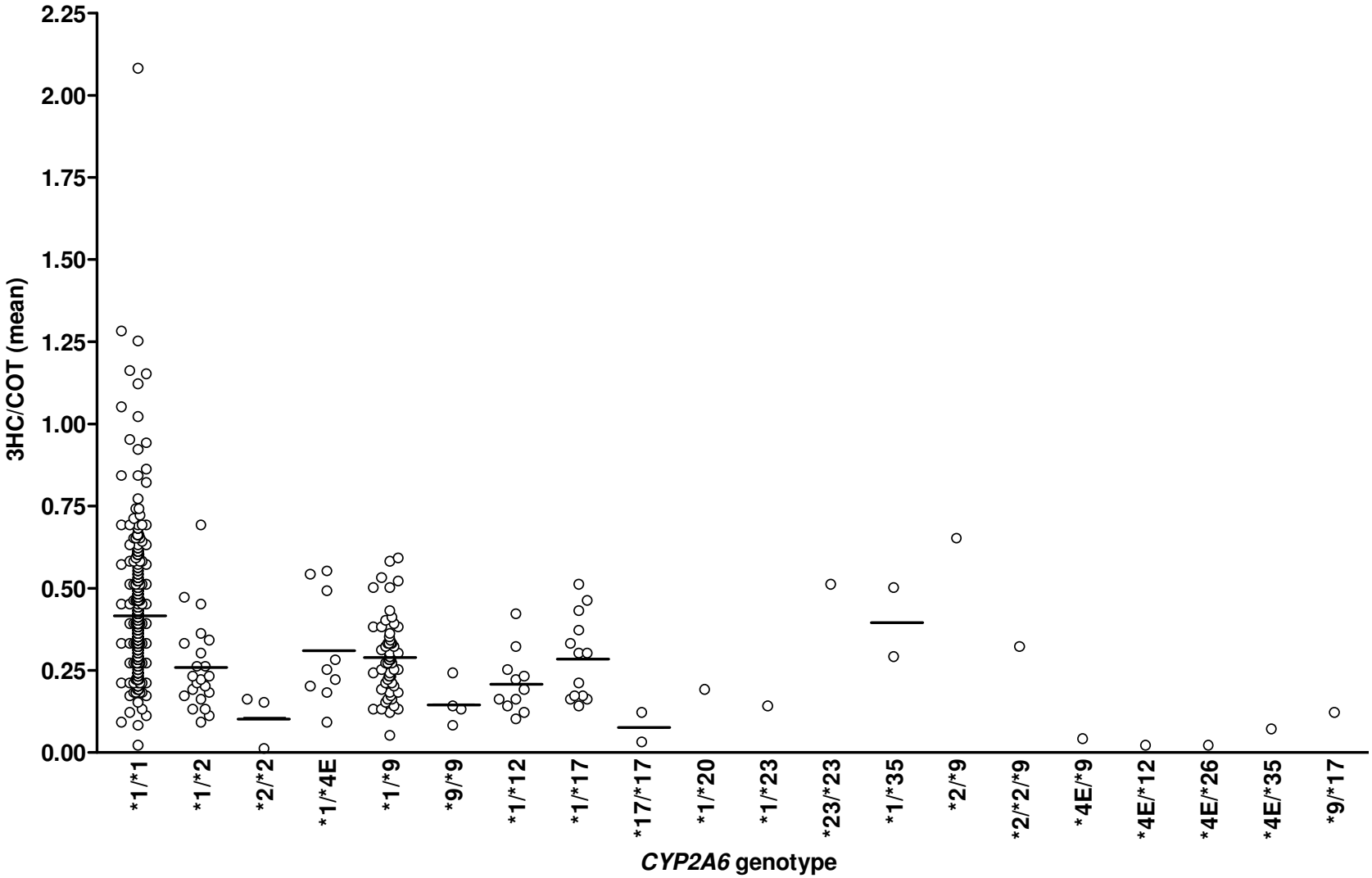


Figure 3.2: 3HC/COT ratios by *CYP2A6* genotype among all participants (n=556). Each dot represents the pre-log transformed 3HC/COT ratio for an individual, and the line indicates the mean 3HC/COT ratio for genotypes with two or more individuals. Refer to Table 3.2 for mean 3HC/COT ± SD values and ANOVA p-values for comparisons between variant genotypes and *CYP2A6*1/*1* (reference group).

mean 3HC/COT was significantly lower for genotypes with *CYP2A6*2* (ANOVA $p<0.0001$), *CYP2A6*4* (ANOVA $p<0.02$), *CYP2A6*9* (ANOVA $p<0.0001$), *CYP2A6*12* (t-test $p<0.0001$), and for those with two or more different variant alleles (t-test $p<0.0001$). Among the Black population, mean 3HC/COT was significantly lower for genotypes with *CYP2A6*9* (t-test $p<0.0001$), *CYP2A6*17* (ANOVA $p<0.0001$), and for those with two or more different variant alleles (t-test $p<0.0001$). However, 3HC/COT ratios for individuals with *CYP2A6*23* and *CYP2A6*35* variants did not differ significantly from CYP2A6*1/*1 individuals (t-test $p>0.5$).

The mean 3HC/COT ratio ± SD for each *CYP2A6* genotype group with all subjects (n=556) was 0.42 ± 0.20 for NMs, 0.28 ± 0.12 for IMs, 0.25 ± 0.16 for SMs, and 0.26 ± 0.14 for RMs (Figure 3.3). For statistical analysis, the ratios were log transformed to obtain a normal distribution (a scatterplot of log (3HC/COT + 1) ratios for each *CYP2A6* genotype group is shown in Figure 3.4) and ANOVA followed by Bonferroni's multiple comparison tests were used to compare the three groups. NMs had significantly higher 3HC/COT compared to IMs ($p<0.001$), SMs ($p<0.001$), and RMs ($p<0.001$), and IMs had modestly, but significantly higher 3HC/COT compared to SMs ($p<0.05$). This is consistent with findings by Ho *et al.* (2009). However, Malaiyandi *et al.* (2006) detected significant differences in ratio only between NMs and SMs, which may have been due to their smaller sample size composed of only Caucasians (n=394) and therefore fewer SMs and IMs (although, analysis among our Caucasian population (n=471) also revealed significant differences between NM vs IMs, NMs vs SMs and NMs vs RMs ($p<0.001$).

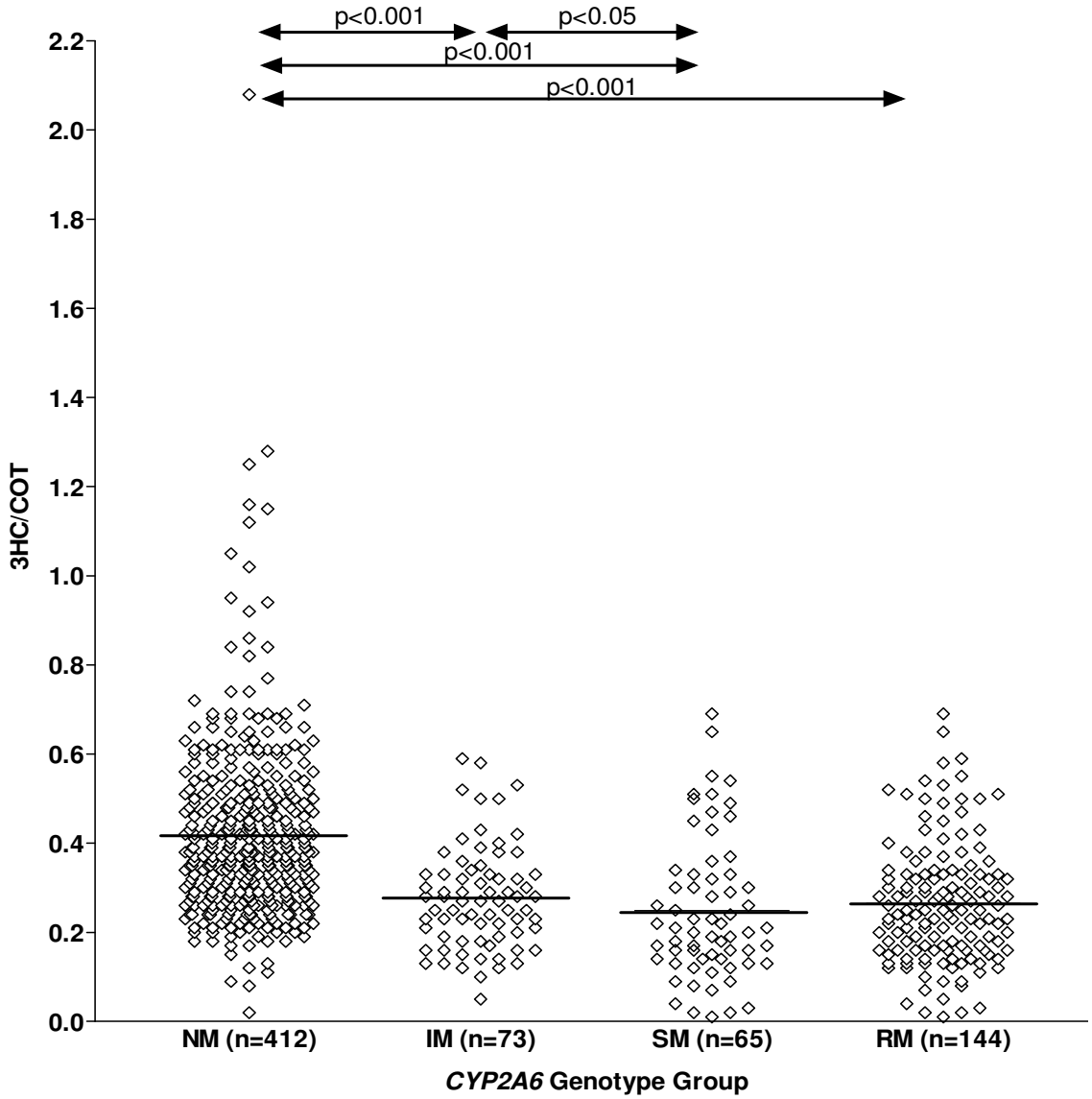


Figure 3.3: Among all participants (n=556), NMs had higher pre-log transformed 3HC/COT compared to IMs, SMs, and RMs, while IMs had modestly higher pre-log transformed 3HC/COT compared to SMs. Each dot represents the pre-log transformed 3HC/COT ratio for an individual, and the line represents the mean 3HC/COT ratio for each genotype group. Mean 3HC/COT ± SD for all genotype groups was 0.42 ± 0.20 for NMs, 0.28 ± 0.12 for IMs, 0.25 ± 0.16 for SMs, and 0.26 ± 0.14 for RMs. For statistical analysis, the ratios were log-transformed to obtain normal distribution. NMs had higher 3HC/COT compared to IMs ($p<0.001$), SMs ($p<0.001$), and RMs ($p<0.001$), and IMs had higher 3HC/COT compared to SMs ($p<0.01$). Among Caucasians (n=471; not shown separately in this figure), mean 3HC/COT ± SD was 0.42 ± 0.20 for NMs (n=362), 0.28 ± 0.12 for IMs (n=64), 0.25 ± 0.16 for SMs (n=40), and 0.26 ± 0.13 for RMs (n=109), with statistical differences between NMs vs IMs ($p<0.001$), NMs vs SMs ($p<0.001$), NMs vs RMs ($p<0.001$), and IMs vs SMs ($p<0.05$). ANOVA followed by Bonferroni's multiple comparison tests were used to compare groups.

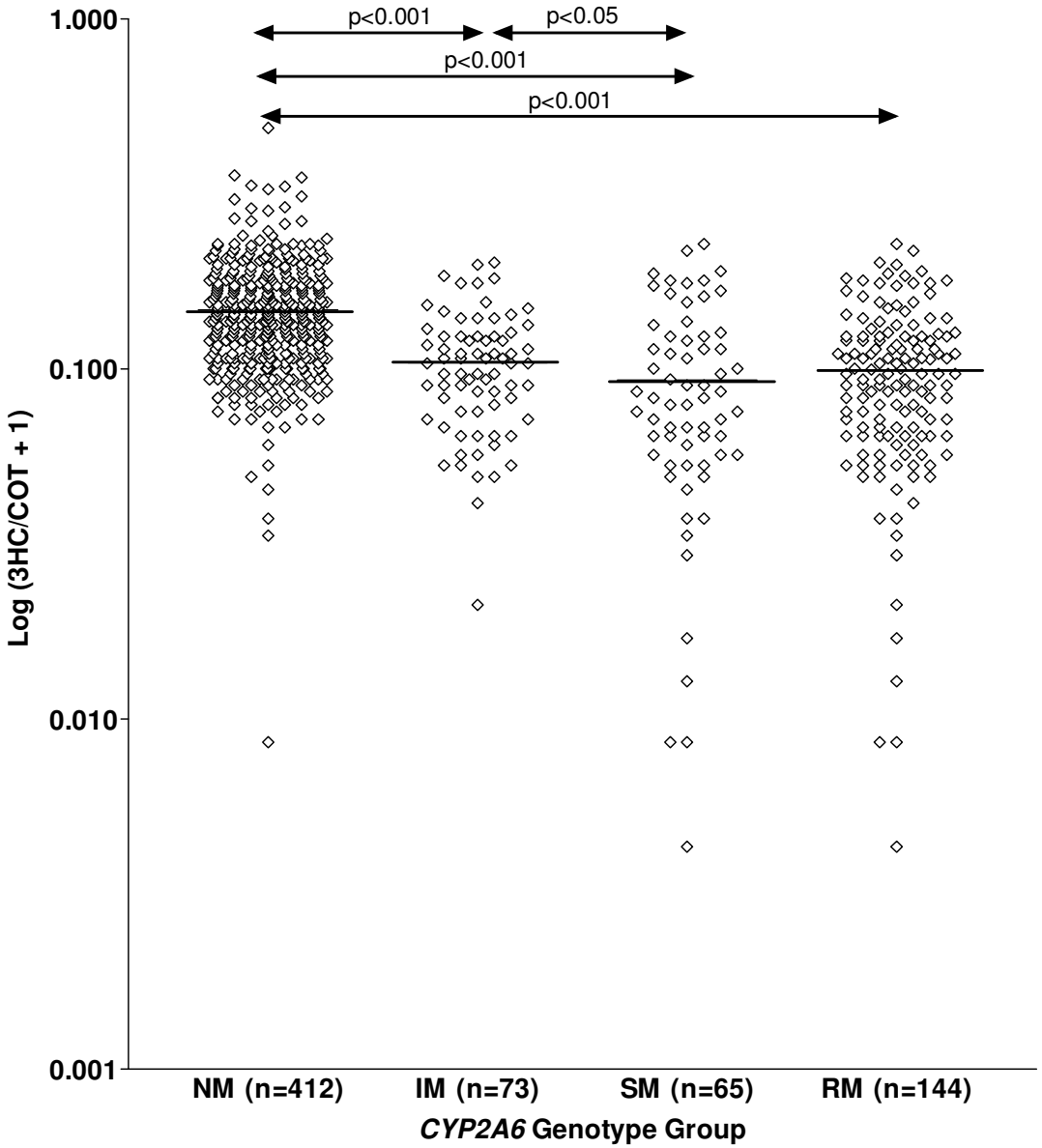


Figure 3.4: Among all participants (n=556), NMs had higher log transformed 3HC/COT compared to IMs, SMs, and RMs, while IMs had modestly higher log transformed 3HC/COT compared to SMs. Each dot represents the log (3HC/COT + 1) ratio for an individual, and the line represents the mean log (3HC/COT + 1) ratio for each genotype group. Mean log (3HC/COT + 1) ± SD for all genotype groups was 0.15 ± 0.05 for NMs, 0.10 ± 0.04 for IMs, 0.09 ± 0.05 for SMs, and 0.10 ± 0.05 for RMs. Log (3HC/COT) values were used for statistical analysis. NMs had significantly higher 3HC/COT compared to IMs ($p<0.001$), SMs ($p<0.001$), and RMs ($p<0.001$), and IMs had significantly higher 3HC/COT compared to SMs ($p<0.05$). Among Caucasians (n=471; not shown separately in this figure), mean log (3HC/COT + 1) ± SD was 0.15 ± 0.05 for NMs (n=362), 0.10 ± 0.04 for IMs (n=64), 0.09 ± 0.05 for SMs (n=40), and 0.10 ± 0.04 for RMs (n=109), with statistical differences between NMs vs IMs, NMs vs SMs, and NMs vs RMs ($p<0.001$). ANOVA followed by Bonferroni's multiple comparison tests were used to compare groups.

3.4 Smoking Variables

3.4.1 Impact of 3HC/COT

The association of 3HC/COT with baseline smoking variables within this study's smoking population has been published (Schnoll *et al.* 2009). The baseline log-3HC/COT ratio was significantly correlated with baseline CPD ($r=0.11$, $p=0.01$) and baseline plasma nicotine ($r=-0.21$, $p<0.001$), but not to baseline nicotine dependence scores with FTND ($r=0.04$, $p>0.05$). These findings agree with previous reports (Lerman *et al.* 2006). In addition, baseline log-3HC/COT did not correlate with number of previous quit attempts ($r=0.04$, $p>0.05$).

3.4.2 Impact of *CYP2A6* Genotype

Smoking variables for all genotype groups were compared via ANOVA followed by Bonferroni's multiple comparisons tests. Among all participants (n=557) , the number of CPD at baseline (mean ± SD) was 21.7 ± 9.4 for NMs, 21.6 ± 9.2 for IMs, 17.9 ± 7.8 for SMs, and 20.0 ± 8.8 for RMs (Figure 3.5). Consistent with previous studies (Rao *et al.* 2000; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006), SMs smoked significantly fewer CPD compared to NMs ($p<0.01$). Among our Caucasian population (n=473), SMs also smoked fewer CPD than NMs, however this difference was not significant (shown in Table 3.8). FTND scores (mean ± SD) among all participants was 5.3 ± 2.1 for NMs, 5.4 ± 2.0 for IMs, 4.8 ± 2.1 for SMs, and 5.1 ± 2.1 for RMs (shown in Table 3.7). Similar FTND scores were found in Caucasians (shown in Table 3.8). Consistent with previous findings, there were no differences in mean scores between genotype groups (ANOVA $p=0.15$) (Malaiyandi *et al.* 2006).

Among all participants (n=557), there were no significant differences in baseline plasma nicotine and COT levels between genotype groups (ANOVA $p=0.06$ and $p=0.44$, respectively), however NMs had significantly higher baseline 3HC levels compared to IMs ($p<0.001$), SMs

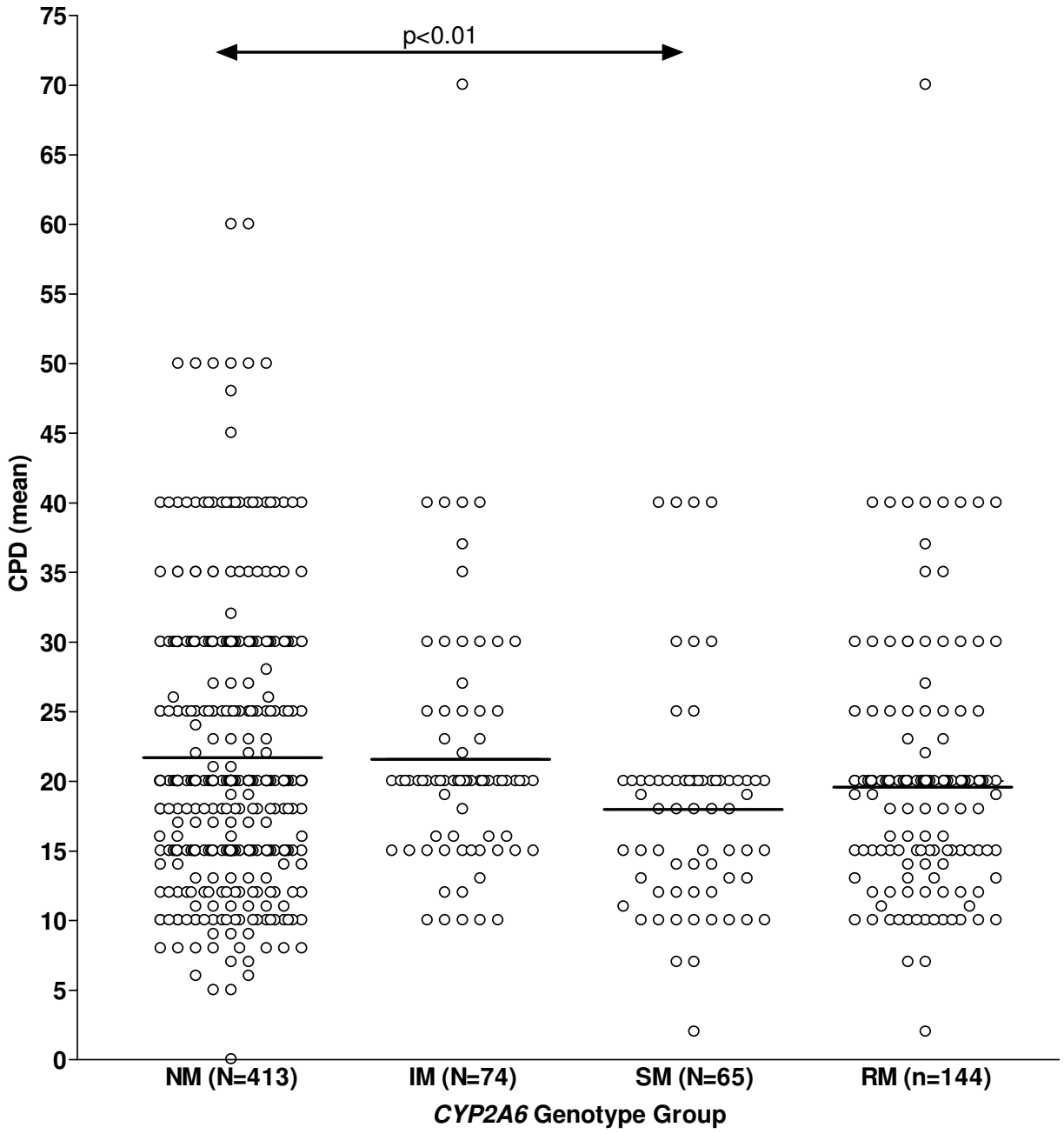


Figure 3.5: Among all participants (n=557), NMs smoked more CPD at baseline compared to SMs. Each dot represents number of CPD smoked for an individual, and the line represents the mean CPD for each genotype group. Mean CPD ± SD was 21.7 ± 9.4 for NMs, 21.6 ± 9.2 for IMs, 18.0 ± 7.8 for SMs, and 20.0 ± 8.8 for RMs. SMs smoked significantly fewer CPD compared to NMs ($p<0.01$). Among Caucasians (n=473; not shown separately in this figure), mean CPD ± SD was 22.4 ± 9.3 for NMs (n=363), 21.0 ± 7.2 for IMs (n=65), 20.2 ± 8.8 for SMs (n=40), and 20.8 ± 7.9 for RMs (n=110), with no statistical differences between genotype groups. ANOVA followed by Bonferroni's multiple comparison tests were used to compare groups.

(p<0.001), and RMs (p<0.001) (shown in TABLE 3.7). Similar differences were found in Caucasians (for nicotine levels, ANOVA p=0.02 but post-hoc analyses did not reveal any group differences; shown in Table 3.8). The differences between NMs, IMs and SMs are consistent with previous findings by Malaiyandi *et al*. (2006).

When differences in age of smoking initiation (mean ± SD) was compared between genotype groups, NMs had a higher age compared to RMs (p<0.05) among all participants (shown in Table 3.7) and among Caucasians (shown in Table 3.8). Previous studies did not find differences in age of initiation between genotype groups (Malaiyandi *et al.* 2006).

3.5 Treatment Variables

3.5.1 Impact of 3HC/COT

The association of 3HC/COT with nicotine patch treatment variables was assessed after one week of treatment and the findings have been published (Schnoll *et al.* 2009). Among week 1 abstainers only (carbon monoxide ≤ 10 ppm), the baseline log-3HC/COT ratio was related to plasma nicotine levels obtained from nicotine patch (r=-0.17, p<0.01). This finding is consistent with findings by Lerman *et al.* (2006). At week 1 of patch treatment, log-3HC/COT ratio was not associated with patch use (r=0.07, p>0.05), patch-related side-effects (r=-0.02, p>0.05), nicotine withdrawal (r=-0.02, p>0.05), nicotine craving (r=-0.02, p>0.05), negative affect (r=0.02, p>0.05) or positive affect (r=-0.03, p>0.05). A previous study found that log-3HC/COT was significantly associated with intensity of craving for cigarettes, but not with patch use, side-effects, or withdrawal symptoms during nicotine patch treatment (Lerman *et al.* 2006).

3.5.2 Impact of *CYP2A6* Genotype

Plasma nicotine and COT levels obtained from patch treatment were measured in all participants at week 1, and only those considered abstinent (carbon monoxide ≤ 10 ppm) were

included in analysis. One Caucasian participant in the IM group (with genotype **1/*12*) had a very high nicotine level of 177 ng/mL (11x higher than the total population mean, 9x higher than and 9 SDs away from the IM mean), most likely due to an error in assessment or contamination, and therefore was removed from the analysis of nicotine levels obtained from patch (once removed, the sample was 23 SDs away from the new IM mean). Plasma nicotine levels obtained from patch treatment were assessed by *CYP2A6* genotype and genotype group among the total population, and separately among Caucasians and Blacks. Plasma cotinine levels obtained from patch treatment were assessed only be genotype group among the total population and Caucasians.

The plasma nicotine levels obtained from patch (mean ± SD) for each *CYP2A6* genotype and genotype group is summarized among all participants (Table 3.10), and separately among Caucasians (Table 3.11) and Blacks (Table 3.12). ANOVA or student's t-test was used to compare the mean nicotine levels for each variant genotype to the *CYP2A6*1/*1* group. ANOVA followed by Bonferroni's multiple comparisons test was used to compared nicotine levels between genotype groups. Among all participants (n=374), the mean nicotine levels were significantly higher for genotypes with *CYP2A6*2* (ANOVA p=0.03) and CYP2A6*17 (t-test p<0.0001). Among genotype groups (n=378), plasma nicotine levels (mean ± SD) for NMs, IMs, SMs and RMs were 15.3 ± 7.8 ng/mL, 16.9 ± 7.1 ng/mL, 20.9 ± 10.2 ng/mL, and 18.7 ± 8.7 ng/mL, respectively (displayed in Figure 3.6). NMs had significantly lower plasma nicotine levels compared to SMs (p<0.001) and RMs (p<0.01). Among Caucasians (n=321), the mean nicotine levels obtained from patch were higher for genotypes with *CYP2A6*2*, *CYP2A6*4*, *CYP2A6*9*, *CYP2A6*12,* and those with two or more variant alleles (only *CYP2A6*4/*9* and *CYP2A6 *4/*12*), with significant differences only for genotypes with two or more variants

Table 3.10: The mean plasma nicotine ± SD (ng/mL) by *CYP2A6* genotype among all abstinent participants at week 1(n=374). The mean plasma nicotine level for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group. The mean plasma nicotine for each genotype group was calculated as a percentage of the NM group.

Allele	Genotype	Frequency (n)	Mean nicotine (ng/mL)	SD (ng/mL)	% Mean	p-value[a]
Reference	*1/*1	273	15.3	7.8	100%	-
*CYP2A6*2*	*1/*2	14	20.4	10.9	134%	0.03
	*2/*2	3	20.6	4.1	135%	
*CYP2A6*4*	*1/*4	8	15.8	9.4	103%	0.85
*CYP2A6*9*	*1/*9	45	16.7	7.1	109%	0.42
	*9/*9	3	18.5	4.7	121%	
*CYP2A6*12*	*1/*12	9	18.0	7.7	118%	0.30
*CYP2A6*17*	*1/*17	9	28.6	8.9	187%	<0.0001
	*17/*17	1	27.2	-	178%	
*CYP2A6*20*	*1/*20	1	12.7	-	83%	-
*CYP2A6*23*	*1/*23	1	14.6	-	96%	-
*CYP2A6*35*	*1/*35	1	17.4	-	114%	-
Two or more different variant alleles	*2/*9	1	13.2	-	86%	0.10
	*4/*9	1	49.1		322%	
	*4/*12	1	19.3		126%	
	*4/*35	1	12.2	-	80%	
	*9/*17	1	17.8	-	117%	
	*2/*2/*9	1	12.8	-	84%	
						p-value[b]
Genotype Group	NM	273	15.3	7.8	100%	-
	IM	54	16.9	7.1	111%	>0.05
	SM	47	20.9	10.2	137%	<0.001
	RM	105	18.7	8.8	122%	<0.01

[a] ANOVA or student's t-test were used to compare the mean nicotine from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with the *CYP2A6*17* allele were grouped for analysis, as were individuals with two or more different variant alleles.

[b] ANOVA followed by Bonferroni's multiple comparisons test was used to compare mean nicotine between genotype groups. The post-hoc p-value for comparisons to the NM group was reported in the table.

Table 3.11: The mean plasma nicotine ± SD (ng/mL) by *CYP2A6* genotype among abstinent Caucasians at week 1 (n=321). The mean plasma nicotine for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group. The mean plasma nicotine for each genotype group was calculated as a percentage of the NM group.

Allele	Genotype	Frequency (n)	Mean nicotine (ng/mL)	SD (ng/mL)	% Mean	p-value[a]
Reference	*1/*1	243	15.0	7.3	100%	-
*CYP2A6*2*	*1/*2	13	18.7	9.1	125%	0.09
	*2/*2	3	20.6	4.1	138%	
*CYP2A6*4*	*1/*4	8	15.8	9.4	105%	0.76
*CYP2A6*9*	*1/*9	38	17.2	6.9	115%	0.16
	*9/*9	3	18.5	4.7	123%	
*CYP2A6*12*	*1/*12	9	18.0	7.7	120%	0.22
Two or more different variant alleles	*2/*9	1	13.2	-	88%	0.02
	*4/*9	1	49.1	-	328%	
	*4/*12	1	19.3	-	129%	
	*2/*2/*9	1	12.8	-	85%	
						p-value[b]
Genotype Group	NM	243	15.0	7.3	100%	-
	IM	47	17.3	7.0	116%	>0.05
	SM	30	18.6	9.7	124%	>0.05
	RM	81	17.8	8.0	119%	<0.05

[a] ANOVA or student's t-test were used to compare the mean nicotine from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with two or more different variant alleles were grouped for analysis.
[b] ANOVA followed by Bonferroni's multiple comparisons test was used to compare mean nicotine between genotype groups. The post-hoc p-value for comparisons to the NM group was reported in the table.

Table 3.12: The mean plasma nicotine ± SD (ng/mL) by *CYP2A6* genotype among abstinent Blacks at week 1 (n=53). The mean plasma nicotine for each genotype was calculated as a percentage of the *CYP2A6*1/*1* group. The mean plasma nicotine for each genotype group was calculated as a percentage of the NM group.

Allele	Genotype	Frequency (n)	Mean nicotine (ng/mL)	SD (ng/mL)	% Mean	p-value[a]
Reference	*1/*1	29	17.8	11.4	100%	-
*CYP2A6*2*	*1/*2	1	42.8	-	241%	-
*CYP2A6*9*	*1/*9	7	13.9	8.0	78%	0.53
	*9/*9	1	22.5	-	124%	
*CYP2A6*17*	*1/*17	9	28.6	8.9	161%	0.01
	*17/*17	1	27.2	-	153%	
*CYP2A6*20*	*1/*20	1	12.7	-	71%	-
*CYP2A6*23*	*1/*23	1	14.6	-	82%	-
*CYP2A6*35*	*1/*35	1	17.4		98%	-
Two or more different variant alleles	*4/*35	1	12.2	-	69%	0.74
	*9/*17	1	17.8	-	100%	
						p-value[b]
Genotype Group	NM	29	14.1	16.0	100%	-
	IM	7	24.4	12.0	174%	>0.05
	SM	17	23.4	9.2	166%	>0.05
	RM	24	23.7	9.8	168%	<0.05

[a] ANOVA or student's t-test were used to compare the mean nicotine from each variant genotype to the reference group (composed of only *CYP2A6*1/*1* individuals). Individuals with a *CYP2A6*9* or **17* allele were grouped for analysis, as were individuals with two or more different variant alleles.

[b] ANOVA followed by Bonferroni's multiple comparisons test was used to compare mean nicotine between genotype groups. The post-hoc p-value for comparisons to the NM group was reported in the table.

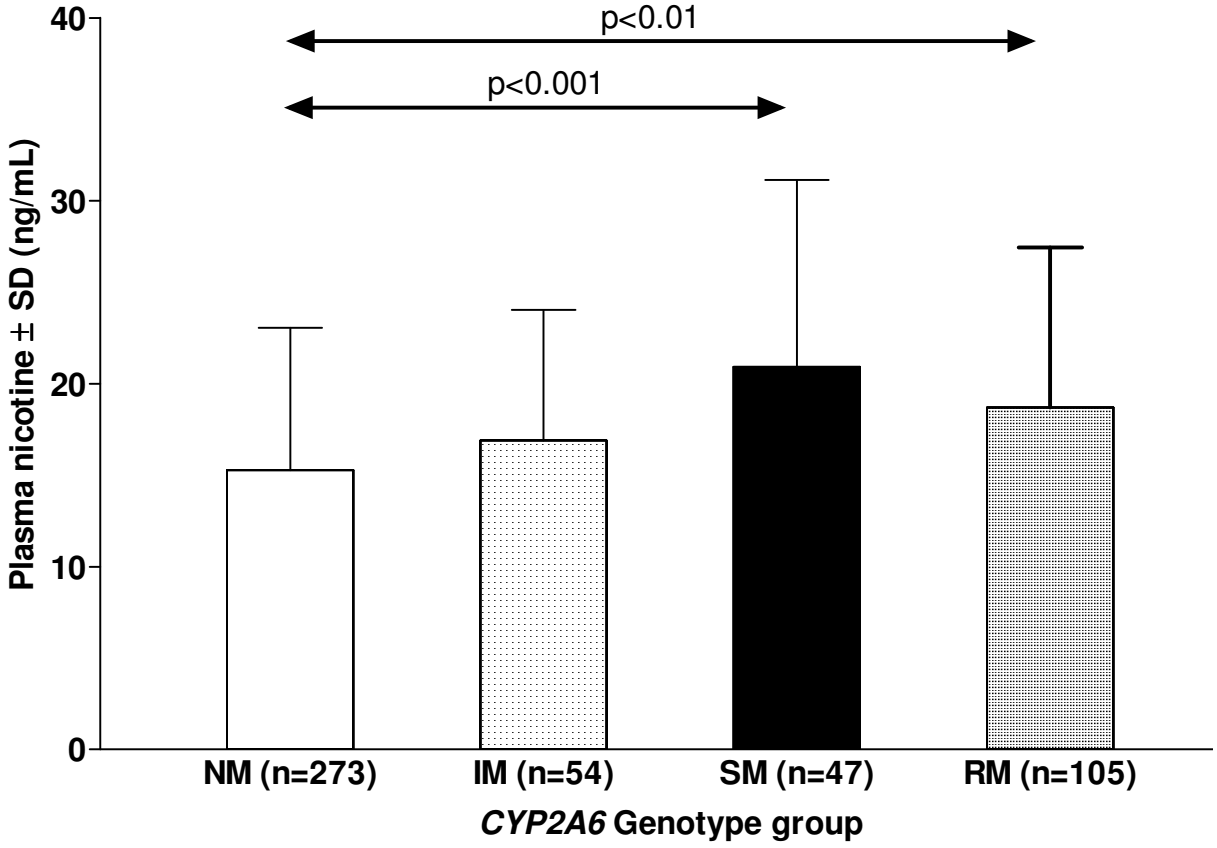


Figure 3.6: Among all abstinent participants at week 1 (n=378), SMs and RMs obtained higher plasma nicotine levels with nicotine patch compared to NMs. Only those considered abstinent (carbon monoxide $\leq$ 10 ppm) were included in analysis. Plasma nicotine levels (mean ± SD) for NMs, IMs, SMs and RMs were 15.3 ± 7.8 ng/mL, 16.9 ± 7.1 ng/mL, 20.9 ± 10.2 ng/mL, and 18.7 ± 8.7 ng/mL, respectively. Plasma nicotine levels differed significantly between NMs vs SMs ($p<0.001$), and NMs vs RMs ($p<0.01$). Among Caucasians (n=324; not shown separately in this figure), plasma nicotine levels (mean ± SD) were 15.0 ± 7.3 ng/mL for NMs (n=243), 17.3 ± 7.0 ng/mL for IMs (n=47), 18.6 ± 9.7 ng/mL for SMs (n=30), and 17.8 ± 8.0 ng/mL for RMs (n=81), with statistical differences between NMs vs RMs ($p<0.05$). ANOVA followed by Bonferroni's multiple comparison tests were used to compare groups.

(p=0.02) (Table 3.11). Among genotype groups (n=324), plasma nicotine levels were 15.0 ± 7.3 ng/mL for NMs, 17.3 ± 7.0 ng/mL for IMs, 18.6 ± 9.7 ng/mL for SMs, and 17.8 ± 8.0 ng/mL for RMs, where NMs had lower plasma nicotine levels compared to RMs ($p<0.05$). These findings for our Caucasian population are in contrast to Malaiyandi *et al.* (2006), where SMs had significantly higher nicotine levels compared to NMs. Among our Black population (n=53), the mean nicotine levels were higher for *CYP2A6*1/*2*, *CYP2A6*9/*9*, and genotypes with *CYP2A6*17*, with significant differences only for the *CYP2A6*17* genotypes (Table 3.12). When analyzed between genotype groups (n=53), plasma nicotine levels were 14.1 ± 16.0 ng/mL for NMs, 24.4 ± 12.0 ng/mL for IMs, 23.4 ± 9.2 ng/mL for SMs, and 23.7 ± 9.8 ng/mL for RMs, where NMs had lower plasma nicotine levels compared to RMs ($p<0.05$).

Plasma COT levels were assessed between genotype groups, and ANOVA followed by Bonferroni's multiple comparisons test was used for comparisons. Among the total population (n=380), the COT levels obtained from patch (mean ± SD) for NMs, IMs, SMs and RMs were 199.3 ± 88.8 ng/mL, 234.6 ± 110.6 ng/mL, 264.5 ± 175.0 ng/mL, and 249.7 ± 141.4 ng/mL, respectively (Figure 3.7). NMs had significantly lower COT levels compared to SMs ($p<0.01$) and RMs ($p<0.001$). Among Caucasians (n=326), plasma COT levels were 193.5 ± 83.1 ng/mL for NMs, 227.1 ± 88.4 ng/mL for IMs, 213.8 ± 103.9 ng/mL for SMs, and 225.0 ± 93.0 ng/mL for RMs. Unlike for all participants, Caucasian NMs had lower COT levels only compared to RMs ($p<0.05$). Our findings for COT levels obtained from patch treatment in Caucasians are consistent with previous findings, where Malaiyandi *et al*. (2006) did not find differences in COT levels between genotype groups.

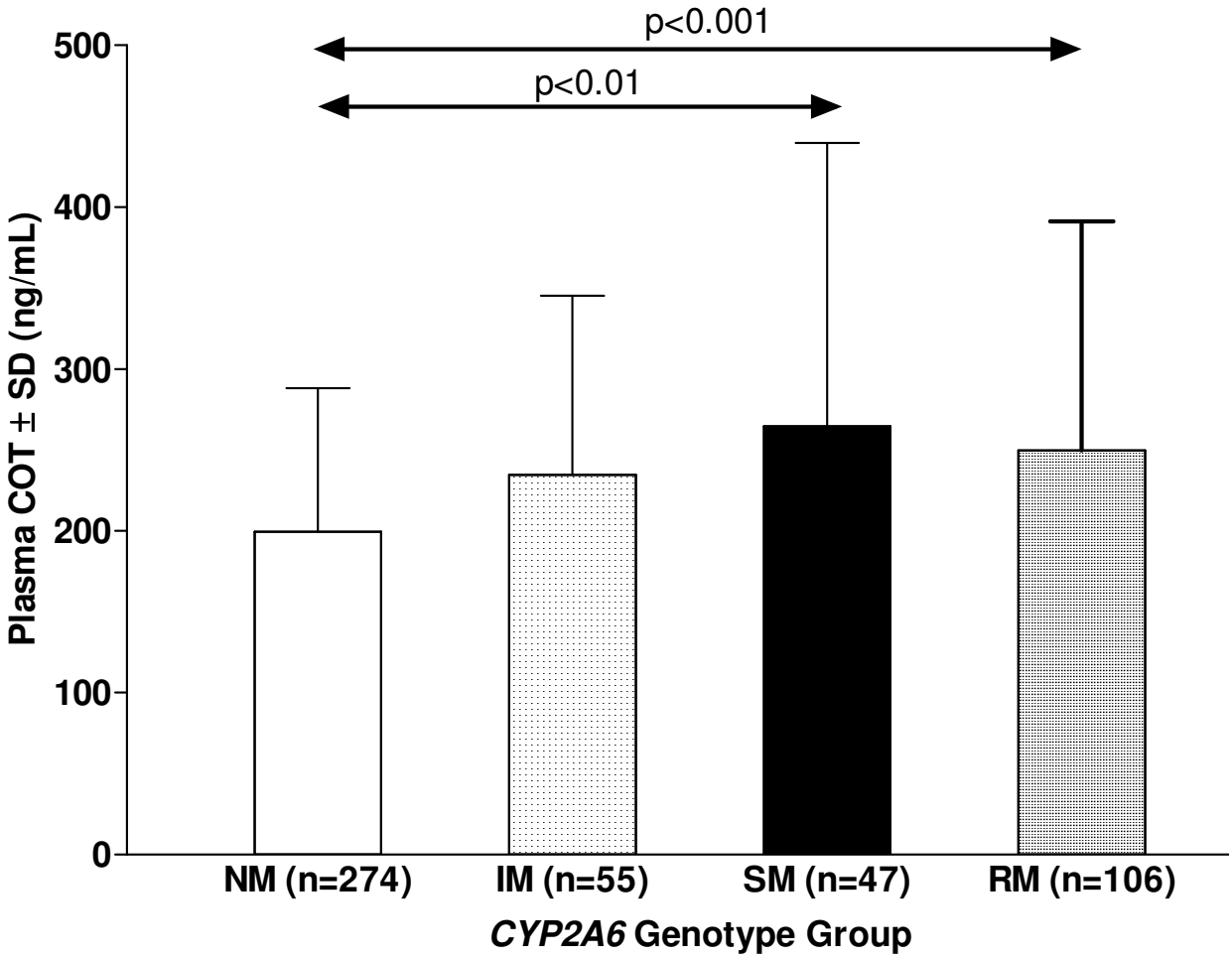


Figure 3.7: Among all abstinent participants at week 1 (n=380), SMs and RMs obtained higher plasma COT levels with nicotine patch compared to NMs. Only those considered abstinent (carbon monoxide $\leq$ 10 ppm) were included in analysis. Plasma COT levels (mean ± SD) for NMs, IMs, SMs and RMs were 199.3 ± 88.8 ng/mL, 234.6 ± 110.6 ng/mL, 264.5 ± 175.0 ng/mL, and 249.7 ± 141.4 ng/mL, respectively. COT levels were significantly higher in SMs versus NMs ($p<0.01$) and RMs versus NMs ($p<0.001$). Among Caucasians (n=326; not shown separately in this figure), plasma COT levels (mean ± SD) were 193.5 ± 83.1 ng/mL for NMs (n=244), 227.1 ± 88.4 ng/mL for IMs (n=48), 213.8 ± 103.9 ng/mL for SMs (n=30), and 225.0 ± 93.0 ng/mL for RMs (n=82), with statistical differences between NMs vs RMs ($p<0.05$). ANOVA followed by Bonferroni's multiple comparison tests were used to compare groups.

3.6 Abstinence rates

All participants in both the standard and extended treatment groups received nicotine treatment for the first 8 weeks. After 8 weeks of nicotine patch therapy, participants in the standard treatment group received 16 weeks of placebo patch, while participants in the extended treatment group continued to receive nicotine patch for 16 weeks. Carbon monoxide-verified point prevalence abstinence for all participants was assessed during treatment at 8 weeks and EOT (24 weeks), and at participant follow-up sessions at 28 weeks and 52 weeks. Participants were counted as abstinent if they reported smoking no cigarettes in the 7 days prior to the given time point, and if they provided a breath sample with a carbon monoxide level of 10 ppm or less.

3.6.1 Overall Abstinence

A time-line of total abstinence rates for both standard and extended treatment among all participants is shown in Figure 3.8. To compare abstinence rates between treatment arms, Chi-tests were used to compute odds ratios and 95% confidence intervals (Table 3.13A for all participants; Table 3.13B for Caucasians). It was hypothesized that participants on extended treatment would have significantly higher EOT (24 weeks) and long-term quit rates compared to participants on standard treatment. When comparing quitting success of the extended treatment group to the standard treatment group among all participants (n=557), there were no differences at week 1 (69.6% vs 68.7%) (OR=1.04, [95% CI: 0.73 – 1.49], p=0.82) or week 8 (35.5% vs 29.9%) (OR=1.29, [95% CI: 0.91 – 1.84], p=0.16), as expected given the identical treatment until week 8. However, EOT (24 weeks) quit rates for extended treatment were significantly higher compared to standard treatment (31.5% vs 20.3%) (OR=1.81, [95% CI: 1.23 – 2.66], p=0.003), as expected given that the standard treatment group had been receiving 16 weeks of placebo patch from week 8 until EOT (24 weeks). Quit rates decreased and no longer remained

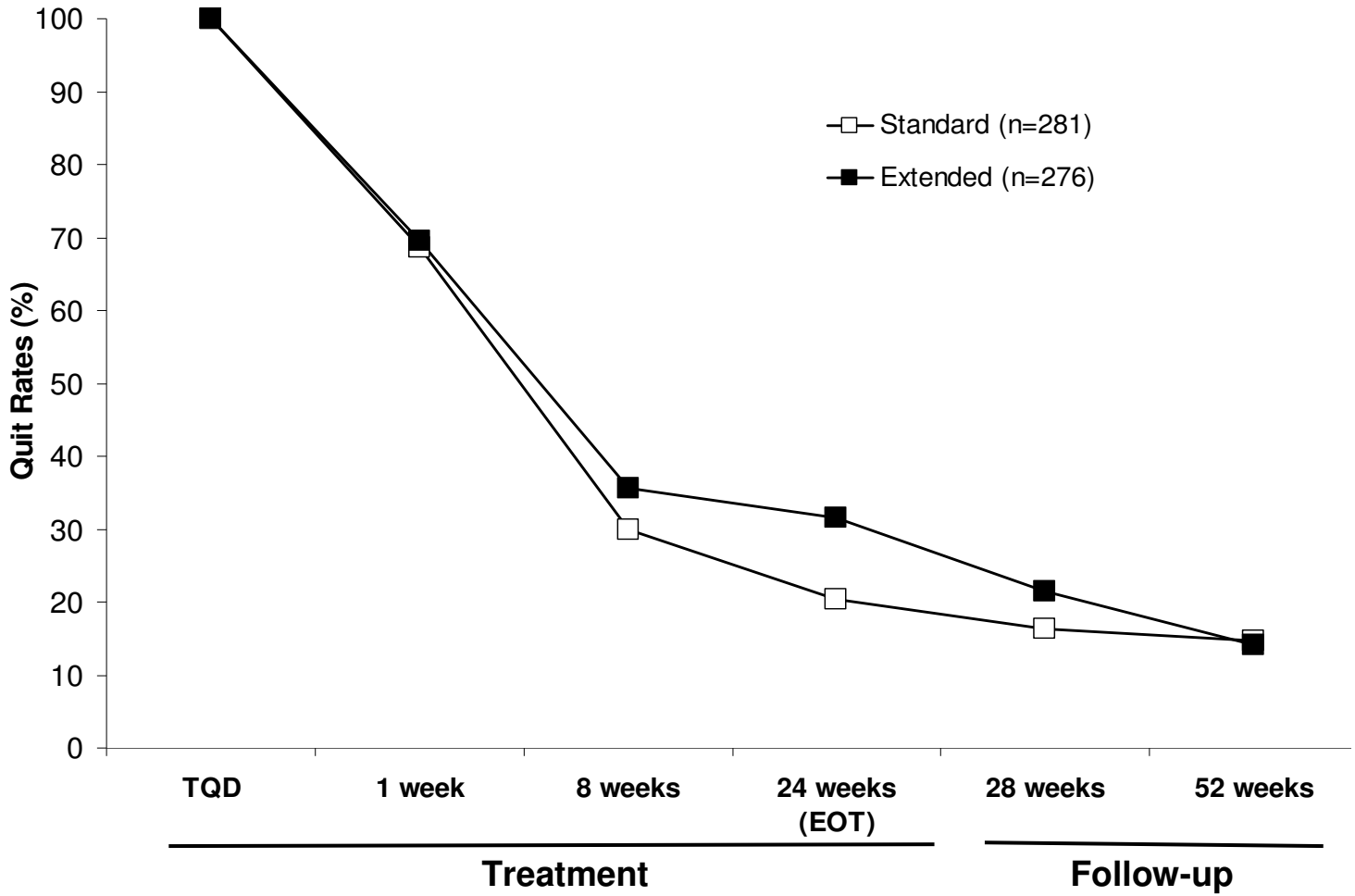


Figure 3.8: Among all participants (n=557), extended nicotine patch therapy increased abstinence rates at 24 weeks, but not at 28 or 52 weeks, compared to standard therapy. Statistical analysis is shown in Table 3.10A. As expected, there were no differences in quit rates at 8 weeks (p=0.16), and quit rates were higher at 24 weeks (EOT) with extended vs standard treatment (p=0.003). At 28 weeks, the difference in quit rates narrowed (p=0.13). There were no long-term differences (52 weeks) (p=0.88).
TQD = target quit date; EOT = end-of-treatment.

Table 3.13A: Odds ratios comparing successful quitting of all smokers (n=557) receiving extended treatment to standard treatment. As expected, there were no differences in quit rates at 8 weeks, but they were higher at 24 weeks (EOT) with extended vs standard treatment. Extended treatment still produced higher quit rates are 28 weeks, but the difference compared to standard treatment was not significant. There were no long-term differences in abstinence at 52 weeks.

Time-point	Odds Ratio	95% Confidence Interval	X^2 test p-value
TQD	-	-	-
1 week	1.04	0.73 – 1.49	0.82
8 weeks	1.29	0.91 – 1.84	0.16
24 weeks (EOT)	**1.81**	**1.23 – 2.66**	**0.003**
28 weeks	1.39	0.91 – 2.13	0.13
52 weeks	0.96	0.60 – 1.55	0.88

TQD = target quit date; EOT = end-of-treatment

Table 3.13B: Odds ratios comparing successful quitting of Caucasian smokers (n=473) receiving extended treatment to standard treatment.

Time-point	Odds Ratio	95% Confidence Interval	X^2 test p-value
TQD	-	-	-
1 week	0.97	0.65 – 1.43	0.86
8 weeks	0.91	0.62 – 1.33	0.63
24 weeks (EOT)	**1.92**	**1.26 – 2.94**	**0.002**
28 weeks	1.43	0.90 – 2.28	0.13
52 weeks	0.91	0.54 – 1.52	0.71

TQD = target quit date; EOT = end-of-treatment

significantly different at 28 weeks (21.4% vs 16.4%) (OR=1.39, [95% CI: 0.91 – 2.13], p=0.13) or 52 weeks (14.1% vs 14.6%) (OR=0.96, [95% CI: 0.60 – 1.55], p=0.88). Similar results were obtained in the Caucasian population (n=473).

3.6.2 Abstinence Rates by 3HC/COT and *CYP2A6* Genotype

The 3HC/COT for all participants was categorized by quartiles of activity (shown earlier in Table 3.9), where those with low CYP2A6 activity represented the 1st quartile (Q1) and those with high CYP2A6 activity represented the 4th quartile (Q4). For purposes of analysis, Q1 abstinence was compared to abstinence of a pooled group of Q2, Q3 and Q4 (pooled due to no differences in abstinence between Q2, Q3 and Q4 at all time-points). To assess abstinence rates between genotype groups, the IMs were pooled with the SMs into a reduced metabolizer (RM) group, and comparisons were made between the RM group and the NM group. The IMs were pooled with SMs because they displayed similar degrees of reduction in 3HC/COT (refer to Figure 3.3), and they had similar levels of nicotine obtained from nicotine patch treatment, suggesting a similar pharmacokinetic impact. In addition, together the IMs and SMs composed 26% of the study population, which is similar to 3HC/COT Q1 (25% of study population). However, the 3HC/COT quartile groupings (Q1 and Q2-Q4) were only similar, but not identical, to the genotype groupings (RM and NM), considering that Q1 was composed of 55% RM and Q2-Q4 was composed of 84% NM (similarly, 53% of RM was in Q1 and 85% of NM was in Q2-Q4).

All participants in both the standard and extended treatment groups received nicotine treatment for the first 8 weeks. Therefore, total 8-week abstinence (standard and extended treatment groups combined) was compared between Q1 vs Q2-Q4 and RM vs NM. Among all participants, Q1 had significantly higher 8-week abstinence (42.3% vs 28.8%) (OR=1.81, [CI:

1.22 – 2.69], p=0.003). This finding has been published in Schnoll *et al.* (2009). Similarly, in our Caucasian population Q1 had significantly higher 8-week abstinence (42.5% vs 27.6%) (OR=1.95, [CI: 1.25 – 3.04], p=0.003), which is consistent with Lerman *et al.* (2006), where abstinence at the end of an 8-week treatment phase in Caucasians was significantly higher for Q1 compared to Q4 (46.3% vs 27.7%). However, when total 8-week abstinence of RMs was compared to total abstinence of NMs among all participants, RMs had higher abstinence than NMs (37.5% vs 31.8%), but the differences were not significant (OR=1.34, [CI: 0.90 – 1.99], p=0.152). Similarly, in our Caucasian population RMs had higher abstinence than NMs (36.3% vs 29.8%), but the differences were not significant (OR=1.35, [CI: 0.86 – 2.11], p=0.19). All further analyses between groups focused on subsequent time points when the treatments diverged.

After 8 weeks of nicotine patch therapy, participants in the standard treatment group received 16 weeks of placebo patch, while participants in the extended treatment group continued to receive nicotine patch for 16 weeks. Abstinence rates for all participants in the extended treatment group, stratified by 3HC/COT quartile and by *CYP2A6* genotype, are shown in Figure 3.9. To compare abstinence rates between 3HC/COT quartiles and between genotype groups, Chi-tests were used to compute odds ratios and 95% confidence intervals (Table 3.14A for all participants; Table 3.14B for Caucasians). It was hypothesized that given extended treatment, participants with slow CYP2A6 activity (i.e. those in Q1 and/or those in the RM group) would have greater quit rates (throughout treatment, at EOT (24 weeks) and follow-up) compared to participants with high CYP2A6 activity (i.e. those in Q2-Q4 and/or those in the NM group). Stratifying by either 3HC/COT or genotype, those with reduced CYP2A6 activity had

higher quit rates than those with normal CYP2A6 activity, reaching significance only in the 3HC/COT groupings. Among all participants, the abstinence rate was significantly higher for

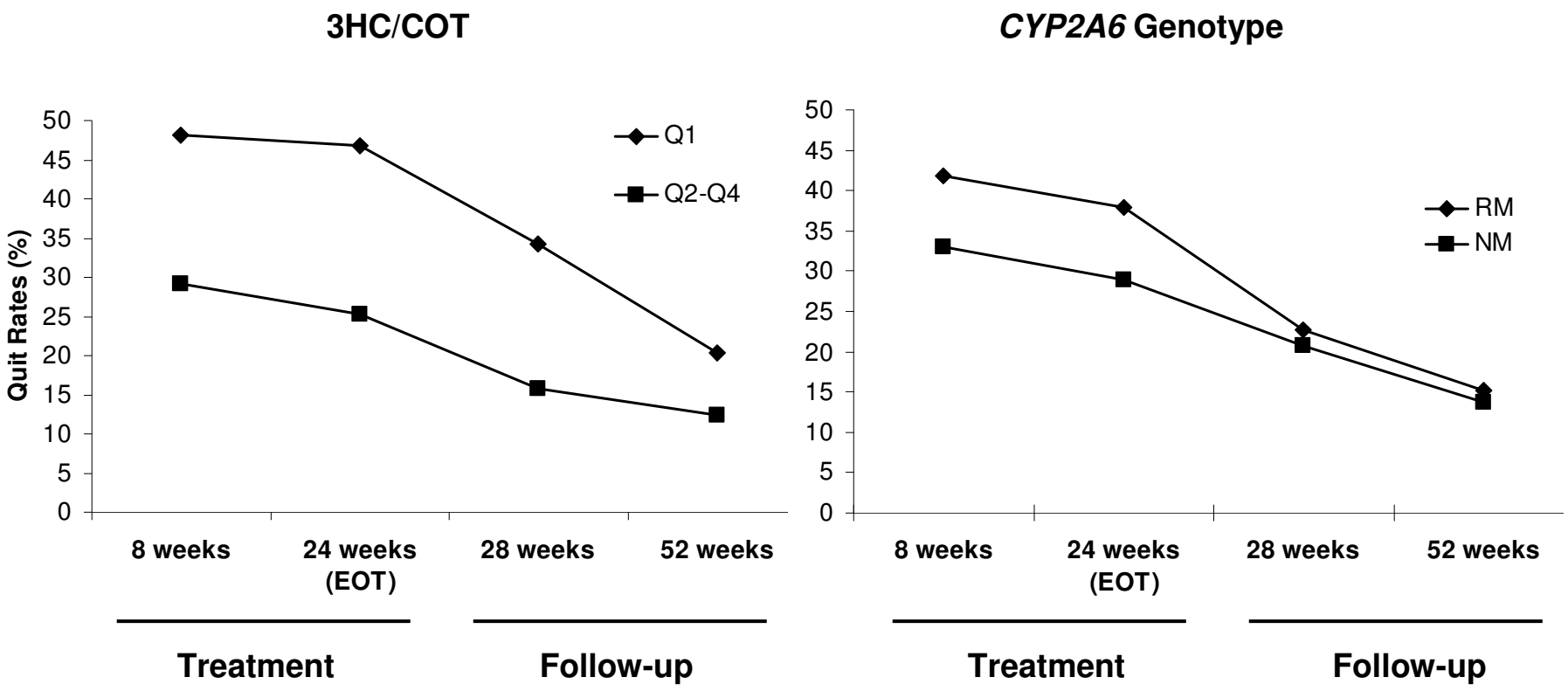


Figure 3.9: Among all participants on *extended* nicotine patch therapy, those in Q1 had higher abstinence rates at weeks 8, 24 (EOT) and 28 compared to those in Q2-Q4. No significant differences were detected between RM and NM groups. Statistical analysis is shown in Table 3.12A. As expected, Q1 had significantly higher abstinence compared to Q2-Q4 at 8 weeks (p=0.003), 24 weeks (EOT) (p=0.001) and 28 weeks (p=0.001). There were no long-term differences at 52 weeks (p=0.09). RMs had higher quit rates at 8 weeks and 24 weeks compared to NMs, but differences were not significant (p>0.10). There were no differences at follow-up (p>0.70).
EOT = end-of-treatment.

Table 3.14A: Odds ratios for successful quitting among all participants on extended patch therapy, comparing Q1 to Q2-Q4, and RMs to NMs. As expected, Q1 had significantly higher abstinence compared to Q2-Q4 at 8 weeks, EOT (24 weeks) and 28 weeks. There were no long-term differences at 52 weeks. RMs had higher quit rates at 8 weeks and EOT (24 weeks) compared to NMs, but differences were not significant. RMs had similar quit rates to NMs at 28 weeks and 52 weeks.

	3HC/COT			***CYP2A6* Genotype**		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	**2.25**	**1.32 – 3.84**	**0.003**	1.46	0.85 – 2.49	0.17
24 weeks (EOT)	**2.61**	**1.51 – 4.50**	**0.001**	1.50	0.87 – 2.60	0.14
28 weeks	**2.76**	**1.52 – 5.02**	**0.001**	1.12	0.60 – 2.11	0.72
52 weeks	1.80	0.90 – 3.59	0.09	1.13	0.54 – 2.36	0.75

EOT = end-of-treatment

Table 3.14B: Odds ratios for successful quitting among Caucasians on extended patch therapy, comparing Q1 to Q2-Q4, and RMs to NMs.

	3HC/COT			***CYP2A6* Genotype**		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	**2.16**	**1.17 – 3.97**	**0.01**	1.35	0.73 – 2.49	0.34
24 weeks (EOT)	**2.79**	**1.51 – 5.14**	**0.0008**	1.79	0.97 – 3.30	0.06
28 weeks	**3.23**	**1.65 – 6.32**	**0.0004**	1.13	0.56 – 2.29	0.73
52 weeks	2.09	0.97 – 4.52	0.06	0.91	0.38 – 2.14	0.82

EOT = end-of-treatment

Q1 versus Q2-Q4 at 8 weeks (48.1% vs 29.2%) (OR=2.25, [CI: 1.32 – 3.84], p=0.003), EOT (24 weeks) (46.8% vs 25.3%) (OR=2.61, [CI: 1.51 – 4.50], p=0.001) and 28 weeks (34.2% vs 15.8%) (OR=2.76, [CI: 1.52 – 5.02], p=0.001). At 52 weeks, abstinence did not differ between Q1 and Q2-Q4 (20.3% vs 12.4%) (OR=1.80, [CI: 0.90 – 3.59], p=0.09). Similar results were obtained in the Caucasian group. Our findings for Q1 vs Q2-Q4 at week 8 were consistent with previous work by Lerman *et al.* (2006) in Caucasians (46.3% for Q1 vs 27.7% for Q4).

When stratified by genotype, abstinence with extended treatment was higher (but did not reach significance) for RMs versus NMs at 8 weeks (41.8% vs 33.0%) (OR=1.46, [CI: 0.85 – 2.49], p=0.17), EOT (24 weeks) (38.0% vs 28.9%) (OR=1.50, [CI: 0.87 – 2.60], p=0.14), 28 weeks (22.8% vs 20.8%) (OR=1.12, [CI: 0.60 – 2.11], p=0.72) or 52 weeks (15.2% vs 13.7%) (OR=1.13, [CI: 0.54 – 2.36], p=0.75) (Table 3.14A). Differences in abstinence were similar in the Caucasian group (Table 3.14B).

Abstinence rates for all participants in the standard treatment group, stratified by 3HC/COT quartile and by *CYP2A6* genotype, are shown in Figure 3.10. To compare abstinence rates between ratio quartiles and between genotype groups, Chi-tests were used to compute odds ratios and 95% confidence intervals (Table 3.15A for all participants; Table 3.15B for Caucasians). Similar to that with extended treatment, it was hypothesized that given standard treatment, participants in Q1 or with SM genotype would have greater quit rates (throughout treatment, at EOT and follow-up) compared to participants in Q2-Q4 or with NM genotype. However, unlike our results for extended treatment, there were no differences in quit rates on standard treatment between 3HC/COT quartiles or between genotype groups at any of the time-points among all participants or among only Caucasians. Our findings for Q1 vs Q2-Q4 (particularly at week 8)

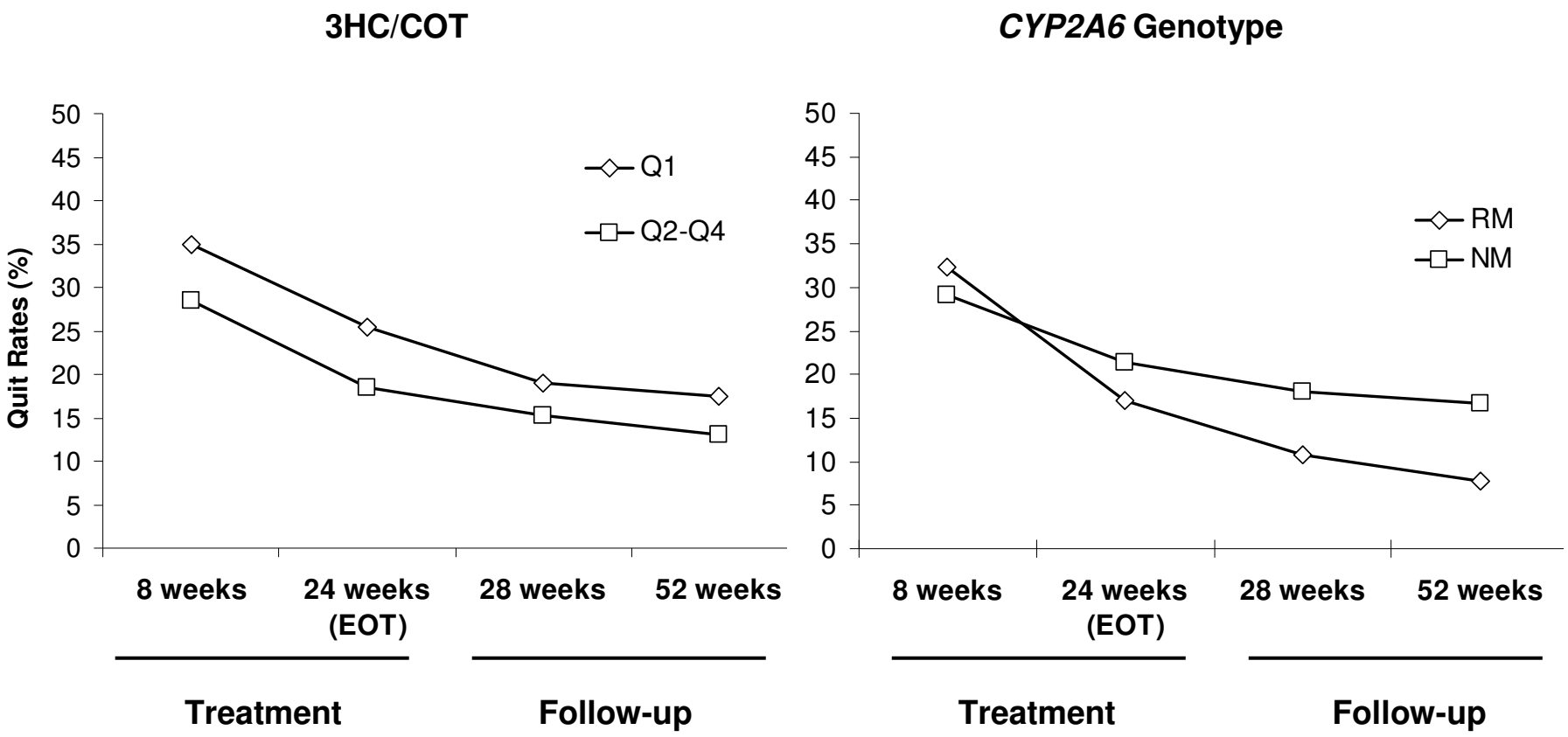


Figure 3.10: Among all participants on *standard* nicotine patch therapy, no significant differences in abstinence rates were detected between Q1 and Q2-Q4, or between RM and NM. Statistical analysis is shown in Table 3.11A. We expected Q1 and RMs to have higher abstinence at all time-points compared to Q2-Q4 and NMs, respectively. Q1 had higher abstinence at all time points compared to Q2-Q4, but the differences were not significant ($p>0.20$). RMs had lower abstinence at EOT (24 weeks) and at follow-up sessions compared to NMs, but the differences were not significant ($p>0.05$).
EOT = end-of-treatment

Table 3.15A: Odds ratios for successful quitting among all participants on standard patch therapy, comparing Q1 to Q2-Q4, and RMs to NMs. Q1 had higher abstinence at all time-points compared to Q2-Q4, however differences were not significant. Aside from 8 weeks, RM had lower abstinence compared to NMs at all time-points, but these differences were not significant.

	3HC/COT			*CYP2A6* Genotype		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	1.35	0.75 – 2.45	0.32	1.16	0.64 – 2.11	0.63
24 weeks (EOT)	1.50	0.78 – 2.91	0.23	0.75	0.36 – 1.56	0.44
28 weeks	1.30	0.63 – 2.69	0.48	0.55	0.23 – 1.29	0.16
52 weeks	1.41	0.66 – 3.01	0.38	0.42	0.16 – 1.11	0.07

EOT = end-of-treatment

Table 3.15B: Odds ratios for successful quitting among Caucasians on standard patch therapy, comparing Q1 to Q2-Q4, and RMs to NMs.

	3HC/COT			*CYP2A6* Genotype		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	1.72	0.89 – 3.31	0.10	1.32	0.68 – 2.57	0.41
24 weeks (EOT)	1.35	0.63 – 2.89	0.44	0.65	0.27 – 1.55	0.32
28 weeks	1.30	0.57 – 2.96	0.53	0.41	0.14 – 1.20	0.09
52 weeks	1.22	0.52 – 2.88	0.65	0.44	0.15 – 1.30	0.13

EOT = end-of-treatment

were inconsistent with previous work by Lerman *et al.* (2006), where abstinence at the end of an 8-week treatment phase was significantly higher for Q1 compared to Q4 (46.3% vs 27.7%).

We also assessed the benefit of extended versus standard patch therapy among those with slow CYP2A6 activity and those with normal CYP2A6 activity. It was hypothesized that all 3HC/COT quartiles and genotype groups would have higher EOT and long-term quit rates with extended treatment versus standard treatment, but that the largest gain in quit rates for extended versus standard treatment would occur among participants with high 3HC/COT (Q2-Q4) and participants in the NM group. To test this hypothesis, abstinence rates for the standard treatment group and extended treatment group were compared among all participants in Q1 and RMs (Figure 3.11, Table 3.16A for all participants; Table 3.16B for Caucasians) and among all participants in Q2-Q4 and NMs (Figure 3.12, Table 3.17A for all participants; Table 3.17B for Caucasians). Chi-tests were used to compute odds ratios and 95% confidence intervals, comparing standard treatment to extended treatment.

At week 8, abstinence was similar between standard and extended treatment groups when stratified by 3HC/COT quartile and *CYP2A6* genotype, which was expected given that all groups received the same treatment until week 8. For all participants in Q1, abstinence rates for extended treatment compared to standard treatment were significantly higher at EOT (24 weeks) (46.8% vs 25.4%) (OR=2.59, [CI:1.26 – 5.31], p=0.01) and 28 weeks (34.2% vs 19.0%) (OR=2.21, [CI: 1.01 – 4.83], p=0.05), indicating that at these times extended treatment was more successful than standard treatment (Table 3.16A). The differences in abstinence between treatment groups were no longer significant at 52 weeks (20.3% vs 17.5%) (OR=1.20, [CI: 0.51 – 2.81], p=0.67). Similar results were obtained in Caucasians, although differences at 28 weeks were marginally non-significant (Table 3.16B).

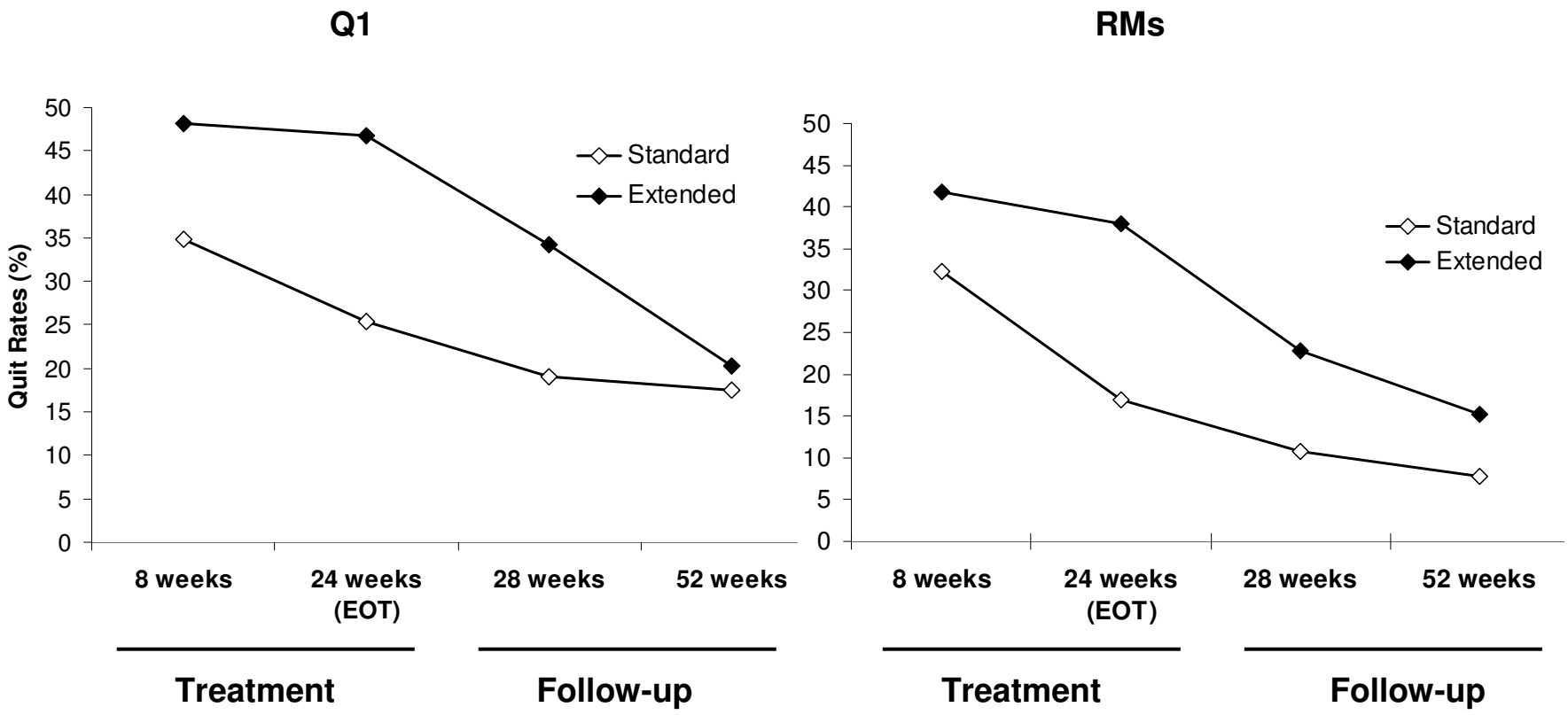


Figure 3.11: Among all participants with slow CYP2A6 activity, extended treatment compared to standard treatment produced higher abstinence rates at 24 weeks and 28 weeks for those in Q1, and at 24 weeks for those with RM genotype. Statistical analyses are shown in Table 3.13A. As expected, Q1 and RMs had significantly higher abstinence with extended vs standard treatment at EOT (24 weeks) (p=0.01 and p=0.01, respectively). At 28 weeks, Q1 and RMs still had higher abstinence on extended vs standard treatment, but differences were only significant for Q1 vs Q2-4 (p=0.04) and not RM vs NM (p=0.06). Contrary to expectations, extended treatment did not increase long-term quit rates for Q1 (p=0.67) or RMs (p=0.17).
EOT = end-of-treatment

Table 3.16A: Odds ratios for successful quitting among all participants in Q1 and RMs, comparing extended treatment to standard treatment. As expected, Q1 and RMs had significantly higher abstinence with extended vs standard treatment at EOT (24 weeks). At 28 weeks, Q1 and RMs still had higher abstinence on extended vs standard treatment, but differences were only significant for Q1 vs Q2-4. Extended treatment did not increase long-term quit rates for Q1 or RMs.

	Q1			RMs		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	1.73	0.87 – 3.41	0.11	1.50	0.76 – 2.98	0.24
24 weeks (EOT)	**2.59**	**1.26 – 5.31**	**0.01**	**3.01**	**1.36 – 6.64**	**0.01**
28 weeks	**2.21**	**1.01 – 4.83**	**0.04**	2.45	0.95 – 6.29	0.06
52 weeks	1.20	0.51 – 2.81	0.67	2.15	0.72 – 6.46	0.17

EOT = end-of-treatment

Table 3.16B: Odds ratios for successful quitting among Caucasians in Q1 and RMs, comparing extended treatment to standard treatment.

	Q1			RMs		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	1.25	0.58 – 2.70	0.57	1.14	0.52 – 2.50	0.74
24 weeks (EOT)	**3.15**	**1.36 – 7.31**	**0.007**	**4.17**	**1.61 – 10.78**	**0.002**
28 weeks	2.51	1.03 – 6.14	0.05	**3.35**	**1.03 – 10.95**	**0.04**
52 weeks	1.38	0.52 – 3.67	0.51	1.70	0.48 – 6.01	0.41

EOT = end-of-treatment

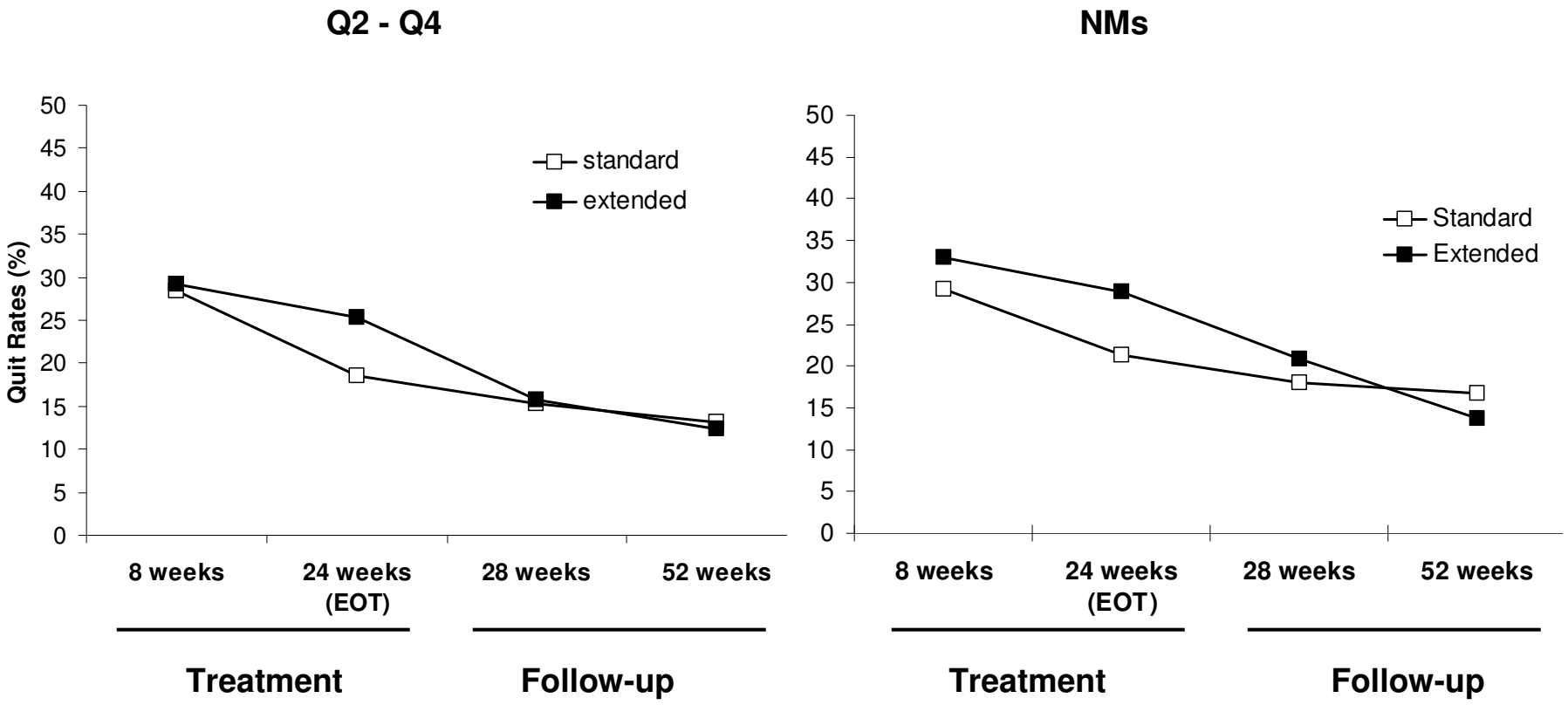


Figure 3.12: Among all participants with normal CYP2A6 activity, there were no differences in abstinence rates between extended treatment and standard treatment for those in Q2-Q4 or those with NM genotype. Statistical analyses are shown in Table 3.14A. We expected higher abstinence with extended treatment at EOT (24 weeks) and follow-up sessions compared to standard treatment, for both Q2-Q4 and NMs. Extended treatment produced higher abstinence than standard treatment at 24 weeks for both Q2-Q4 and NMs, but the differences were not significant ($p>0.09$ and $p>0.07$, respectively). There were no differences in abstinence between treatment groups at follow-up sessions in Q2-Q4 ($p>0.80$) or NMs ($p>0.40$).
EOT=end-of-treatment

Table 3.17A: Odds ratios for successful quitting among all participants in Q2-Q4 and NMs, comparing extended treatment to standard treatment. Extended treatment resulted in higher abstinence compared to standard treatment at EOT (24 weeks) for both Q2-Q4 and NMs, but the differences were not significant. There were no differences in abstinence between treatment groups at follow-up sessions in Q2-Q4 or NMs.

	Q2-Q4			NMs		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	1.04	0.68 – 1.59	0.85	1.20	0.79 – 1.82	0.40
24 weeks (EOT)	1.49	0.94 – 2.37	0.09	1.51	0.96 – 2.36	0.07
28 weeks	1.04	0.62 – 1.76	0.88	1.19	0.73 – 1.94	0.48
52 weeks	0.94	0.53 – 1.67	0.83	0.79	0.46 – 1.37	0.40

TQD=target quit date; EOT=end-of-treatment

Table 3.17B: Odds ratios for successful quitting among Caucasians in Q2-Q4 and NMs, comparing extended treatment to standard treatment.

	Q2-Q4			NMs		
Time-point	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**	**Odds Ratio**	**95% Confidence Interval**	**X^2 test p-value**
8 weeks	0.99	0.63 – 1.57	0.97	1.11	0.71 – 1.75	0.64
24 weeks (EOT)	1.52	0.92 – 2.51	0.10	1.51	0.93 – 2.44	0.10
28 weeks	1.01	0.57 – 1.79	1.00	1.20	0.71 – 2.03	0.51
52 weeks	0.81	0.44 – 1.50	0.50	0.82	0.46 – 1.45	0.49

TQD=target quit date; EOT=end-of-treatment

Similar to Q1, RMs on extended treatment compared to standard treatment had significantly higher abstinence at EOT (24 weeks) (38% vs 16.9%) (OR=3.01, [CI: 1.36 – 6.64], p=0.01) and continued to have higher abstinence at 28 weeks (22.8% vs 10.8%), but this difference did not reach statistical significance (OR=2.45, [CI: 0.95 – 6.29], p=0.06) (Table 3.16A). At 52 weeks, there were no differences in abstinence rate between extended and standard treatment (15.2% vs 7.7%) (OR=2.15, [CI: 0.72 – 6.46], p=0.17). Similar differences were found among Caucasians (Table 3.16B).

Contrary to our hypothesis, all participants in Q2-Q4 and NMs did not show higher quit rates on extended versus standard treatment (Table 3.17A). Although Q2-Q4 and NMs tended to have higher abstinence at EOT (24 weeks) on extended treatment compared to standard treatment (OR=1.49, [CI: 0.94 – 2.37], p=0.09, and OR=1.51, [CI: 0.96 – 2.36], p=0.07, respectively), these differences did not reach significance. There were no differences in quit rates at 28 weeks or 52 weeks. Similar results were obtained in Caucasians (Table 3.17B).

4. DISCUSSION

4.1 Association of *CYP2A6* Genotype with 3HC/COT

The mean 3HC/COT ratio for all genotypes with slow *CYP2A6* variants in the total study population and just in Caucasians were all significantly lower than the reference group (*CYP2A6*1/*1* genotype) (Table 3.2, 3.3). In the Black population, genotypes with *CYP2A6*9*, **17* and with more than one *CYP2A6* variant had lower mean 3HC/COT compared to the *CYP2A6*1/*1* group (Table 3.4). However, Black participants with a *CYP2A6*23* variant (n=2) and those with a *CYP2A6*1/*35* genotype (n=2) did not have different 3HC/COT compared to the reference group. It was specifically one individual with a **23/*23* genotype and one individual with a **1/*35* genotype that had uncharacteristically high 3HC/COT, skewing the mean 3HC/COT for those genotypes. The sole individual with *CYP2A6*1/*2* also had 3HC/COT higher than the reference group. It is possible that these individuals had *CYP2A6* variants that increase CYP2A6 activity, such as the duplication variant *CYP2A6*1x2* (Rao *et al.* 2000) or *CYP2A6*1B* (Yamano *et al.* 1990; Nakajima *et al.* 2001; Pitarque *et al.* 2004). None of the study participants were genotyped for *CYP2A6*1x2* or *CYP2A6*1B*. Perhaps if we genotyped for these variants and separated all individuals with at least one of these variant alleles into a separate genotype category (or placed them into the reference group), we could detect a larger effect of slow *CYP2A6* variants on mean 3HC/COT.

There were also significant differences in the proportion of genotype groups between Blacks and Caucasians (Table 3.5). Compared to Caucasians, the Black population had a considerably larger proportion of SMs (31% vs 12%) and a smaller proportion of NMs (58% vs 79%). This was expected, given that the Black population contains numerous slow *CYP2A6* variants that we assayed (i.e. *CYP2A6*2,*4, *17, *20 *23, *24, *25, *26, *27,* and **35*; or *CYP2A6*9/*9*,

*CYP2A6*9/*12,* and *CYP2A6*12/*12* genotypes), some of which occurred at a relatively high frequency in our study (e.g. 11% allele frequency for *CYP2A6*17*), while the Caucasian population carries fewer slow *CYP2A6* variants (i.e. only *CYP2A6*2* and **4* were genotyped; *CYP2A6*9/*9*, *CYP2A6*9/*12,* and *CYP2A6*12/*12* genotypes were also SM), which occurred at a relatively low frequency (i.e. allele frequencies of ~3% for *CYP2A6*2*, ~1% for *CYP2A6*4*, and ~1% for *CYP2A6*12;* CYP2A6*9 had a high allele frequency of 7%) (Table 3.1). This ethnic difference in the proportion of genotype groups is one reason why we assessed baseline characteristics, smoking variables and treatment outcomes for both the entire study population and for only the Caucasian group. We also chose to analyze the Caucasian group separately for the purpose of comparing our findings to a smoking cessation trial with nicotine patch in Caucasians by Malaiyandi *et al.* (2006), which shared similar inclusion criteria to our study and required treatment-seeking smokers that smoked more than 10 CPD over the past 12 months.

In accordance with previous work by Malaiyandi *et al.* (2006), *CYP2A6* genotype groupings in our study associated with 3HC/COT among our total population and among our Caucasian population. That is, the mean 3HC/COT ratio increased in order from SMs to IMs to NMs, with significant differences between all genotype groups (Figure 3.3). These findings demonstrate that 3HC/COT is a good predictor of *CYP2A6* genotype (Dempsey *et al.* 2004; Benowitz *et al.* 2006b; Malaiyandi *et al.* 2006). Since there was only a modest difference between SMs and IMs, compared to the relatively large difference between NMs and IMs/SMs, it suggests that IMs and SMs have similar pharmacokinetics, and therefore for purposes of analysis of abstinence they were pooled into one genotype group (i.e. RM). Consistent with this, NMs also had significantly higher 3HC/COT compared to RMs in both our total population and Caucasians.

Although the 3HC/COT associated with genotype group, there was large variation in 3HC/COT within each group. Particularly in the NM group, there were several individuals with very low 3HC/COT, which perhaps can be explained by the possibility that they have unknown slow *CYP2A6* variants that were not detected with our present set of genotyping assays. There were also several slow *CYP2A6* variants which we did not assess with our assays because of their very low frequency, such as *CYP2A6*3*, **5*, **6*, **11*, **13* and **15*. Other *CYP2A6* variants were excluded because of their low frequency and/or limited information regarding their CYP2A6 impact, such as *CYP2A6*22, *29, *30, *31, *32, *33, *34, *36,* and **37*. Perhaps if we genotyped for all known slow *CYP2A6* variants, regardless of their low frequency, we could have detected more SMs (otherwise categorized as NMs) and therefore reduced the variability in 3HC/COT seen in the NM group.

In the NM and SM group, there were also several individuals with high 3HC/COT ratios. Perhaps this can be explained by the presence of increased-activity alleles, such as *CYP2A6*1x2* and *CYP2A6*1B*, which we did not genotype for. If we had genotyped all participants for *CYP2A6*1x2* and *CYP2A6*1B* variants, we could have separated all individuals with these variants into a separate genotype group (e.g. fast metabolizers, representing >100% CYP2A6 activity), and left the remaining *CYP2A6*1/*1* individuals in the NM group. Doing this would possibly decrease the variability of 3HC/COT within each genotype group, and potentially result in a larger difference in mean 3HC/COT between genotype groups.

Aside from the limitations of our chosen genotyping assays, there are also environmental influences on CYP2A6 activity that can explain 3HC/COT variability. For instance, drugs such as rifampicin, dexamethasone and phenobarbital increase CYP2A6 activity (Rae *et al.* 2001; Itoh *et al.* 2006; Onica *et al.* 2008). Female participants on contraceptives would also have increased

CYP2A6 activity and nicotine metabolism (Benowitz *et al.* 2006a). On the other hand, drugs such as methoxsalen and tryptamine inhibit CYP2A6 activity (Kharasch *et al.* 2000), and dietary compounds such as grapefruit juice can inhibit CYP2A6-mediated coumarin metabolism (Runkel *et al.* 1997). Furthermore, nicotine itself is an inhibitor of CYP2A6 activity (Denton *et al.* 2004), which could possibly alter CYP2A6 activity according to level of smoking. Differences in pharmacokinetics may also influence apparent CYP2A6 activity, such as urinary pH, or differences in liver blood flow (Cholerton *et al.* 1994; Benowitz *et al.* 1997; Hukkanen *et al.* 2005). Aside from increased metabolism by contraceptives, females also have faster nicotine metabolism compared to males (Benowitz *et al.* 2006a). Although there were no differences in gender proportion between genotype groups, the mixed population of males and females is another potential cause of variability seen in 3HC/COT within each genotype group.

Furthermore, other nicotine metabolizing pathways may cause variation in 3HC/COT within the genotype groups. Although the conversion of COT to 3HC is exclusively by CYP2A6, increased glucuronidation of COT or 3HC may impact an individual's 3HC/COT ratio and apparent CYP2A6 activity. Thus, it is useful to consider the impact of both genotype and phenotype on smoking cessation to determine which is a better predictor of cessation.

4.2 Association of *CYP2A6* genotype with smoking and treatment variables

Among all participants, SMs smoked significantly fewer CPD compared to NMs (18 ± 8 vs 22 ± 9) (Table 3.7, Figure 3.5). RMs also smoked fewer CPD (20 ± 9) compared to NMs, but this difference was not significant, given that IMs smoked a similar number of CPD to NMs (22 ± 9). Among Caucasians, SMs and RMs also smoked fewer CPD than NMs, but these differences did not reach statistical significance (Table 3.8). Malaiyandi *et al.* (2006) reported that SMs smoked fewer CPD than NMs, which is consistent with our findings in the total study population and in

Caucasians (although significant only among the total population). These differences between the total population and the Caucasians only are most likely due to the smoking behaviours of our Black population within the total population. For one, the CPD smoked by SMs in our total population was lower compared to the Caucasian group (18 ± 8 vs 20 ± 9). Furthermore, our Black population smoked fewer CPD than the Caucasian population (17 ± 9 vs 22± 9), where SMs in the Black population smoked 14 ± 4 CPD and SMs in the Caucasian population smoked 20 ± 9 CPD. The lower CPD smoked by the Black population agrees with previous reports suggesting that the majority of African-American smokers are light-smokers (Kandel and Chen 2000). Since the Black population had a higher proportion of SMs compared to Caucasians (31% vs 9%), the impact of the Black population's lighter smoking would reduce the number of CPD smoked by the total study population, particularly in the SM group.

The difference of 4 CPD between the SM and NM groups in our total study population is perhaps less of a difference than what we would see in the general smoking population, due to the possibility that we are excluding a population of SMs that are light smokers and those who are less dependent and have already quit smoking. Our findings are different from Schoedel *et al.* (2004), who reported a difference of 7 CPD between SMs and NMs (21 ± 9 vs 28 ± 15), which was assessed among non-treatment-seeking DSM-IV-dependent Caucasian smokers. Furthermore, in the latter study smokers were defined as individuals who smoked more than 100 cigarettes in their lifetime (i.e. includes light smokers), and had an average age of 36 ± 12 years. In contrast, and in addition to the fact that our study recruited treatment-seeking smokers who smoked more than 10 CPD (i.e. excludes light smokers), the smokers in our study were older (45 ± 10 years) and were not required to meet criteria for nicotine dependence. However, the mean FTND ± SD for our study's population was 5.3 ± 2.1 (with no differences between genotype

groups), which indicates a medium level of dependence (Fagerstrom *et al.* 1990). The differences in age, level of smoking, nicotine dependence, and whether the study population was treatment-seeking or not, most likely contributed to the magnitude of difference in CPD found between SM and NM in our study compared to findings by Schoedel *et al.* (2004).

The levels of nicotine obtained from patch treatment were assessed by *CYP2A6* genotype and by genotype group among abstinent participants. We expected that genotypes with variant alleles would have higher plasma nicotine obtained from patch treatment compared to the *CYP2A6*1/*1* genotype, given that all variant genotypes (aside from those with *CYP2A6*23* or **35*) had significantly lower baseline 3HC/COT (Table 3.2) and therefore slower CYP2A6 activity. However, the association between nicotine levels obtained from patch treatment and *CYP2A6* variant genotypes was not as strong as expected. Among all abstinent participants (Table 3.10), the mean nicotine levels were significantly higher only for genotypes with *CYP2A6*2* or **17* compared to *CYP2A6*1/*1*. However, when comparisons were made between genotype groups, SMs and RMs had higher nicotine levels compared to NMs (shown in Figure 3.6).

Among abstinent Caucasians (Table 3.11), only the group of genotypes with two or more different variants had significantly higher mean nicotine levels compared to *CYP2A6*1/*1*. In contrast to the total study population, the mean nicotine levels obtained from patch in Caucasians were only higher in the RM group compared to NMs. Our finding was inconsistent with Malaiyandi *et al.* (2006), where they found significantly higher nicotine levels obtained from patch treatment in SMs compared to NMs in a Caucasian population. Among abstinent Blacks in our study (Table 3.12), the mean nicotine levels obtained from patch were higher only for genotypes with *CYP2A6*17*, and similar to Caucasians, only RMs had higher nicotine levels compared to NMs.

For all the population groups (total, Caucasians, and Blacks), there were no differences in nicotine levels between IMs and SMs, which suggests similar pharmacokinetics between the two genotype groups and supports our decision to pool them into one metabolizer group (i.e. RM) for the analyses of abstinence. As mentioned, the RM group had significantly higher nicotine levels obtained from patch compared to NMs among all three populations.

The COT levels obtained from nicotine patch treatment were assessed by *CYP2A6* genotype group among all participants and Caucasians. Among the total population and just Caucasians, SMs had higher COT levels compared to NMs, but this difference was only significant among the total population (Figure 3.7). However, among both the total population and Caucasian population, the RM group had significantly higher COT levels obtained from patch compared to NMs. Our findings for similar COT levels between SMs and NMs among our Caucasian population agree with Malaiyandi *et al.* (2006), where they reasoned that the similarity in cotinine levels obtained from nicotine patch treatment between NMs and SMs in their study was due to a balancing of slower conversion of nicotine to COT with the slower conversion of COT to 3HC.

The difference in nicotine and COT levels between SMs and NMs among our total population, but not among our Caucasian group, is influenced by the inclusion of Black participants. As mentioned earlier, our Black population had a considerably larger proportion of SMs (31% vs 12%) and a smaller proportion of NMs (58% vs 79%) compared to Caucasians, given that the Black population contains a larger variety (with high frequency) of slow *CYP2A6* variants. Increasing the proportion of SMs in the total population most likely increased the power of SM genotype on increasing nicotine and COT levels obtained from patch treatment. The differences in COT levels can be explained along the premise that while conversion of COT to

3HC is exclusive to CYP2A6, the metabolism of nicotine to COT is only mediated 80% by CYP2A6 (Benowitz and Jacob 1994; Nakajima *et al.* 1996a; Nakajima *et al.* 1996b; Messina *et al.* 1997; Dempsey *et al.* 2004). Therefore, slower conversion of COT to 3HC is not necessarily balanced by slower conversion of nicotine to COT, especially if other pathways such as glucuronidation compensate for slower CYP2A6 activity. In particular, there are ethnic differences in nicotine and COT glucuronidation, where it has been reported to be polymorphic among Blacks (i.e. slow and fast N-glucuronidation), but unimodal among Caucasians (i.e. fast glucuronidation) (Benowitz *et al.* 1999). These differences contribute to the variation in nicotine and COT metabolism between ethnicities, in addition to *CYP2A6* polymorphisms, and most likely contribute to our different results in terms of nicotine and COT levels obtained for Caucasians and for the total study population (composed of Caucasians and Blacks).

Among the total study population, the higher nicotine and COT levels obtained from patch for SMs versus NMs is inconsistent with baseline levels of nicotine and COT obtained from smoking (Table 3.8), where there were no differences between genotype groups. Although NMs had faster baseline nicotine metabolism (indicated by higher 3HC/COT) compared to SMs, they also smoked more CPD, suggesting that they were titrating their smoking behaviour to maintain optimum brain and plasma nicotine levels and therefore obtaining similar baseline nicotine and COT levels to SMs. Previous studies also suggest NMs take larger puffs compared to SMs (Strasser *et al.* 2007), which would further allow NMs to titrate their nicotine intake per cigarette and to attain similar nicotine and COT levels to that of SMs. Nonetheless, our findings suggest that NMs are experiencing a smaller replacement of nicotine during nicotine patch therapy compared to SMs. Based on this, perhaps we should consider increasing the dose of nicotine for the NMs so that they obtain similar levels of nicotine from patch as SMs (Ebbert *et al.* 2007).

4.3 Efficacy of Standard Versus Extended Treatment

The abstinence rates we obtained for our total study population and for only our Caucasian population were similar when stratified by treatment group and/or 3HC/COT quartiles or *CYP2A6* genotype. Therefore, for the purposes of this section, we discussed abstinence only for the total study population.

The similar quit rates obtained with standard and extended treatment after 8 weeks was expected (30% and 36%, respectively) (Figure 3.8), given that both treatment groups received identical treatment (21mg/day nicotine) for the first 8 weeks. The standard group continued their treatment with placebo patch for 16 weeks, while the extended group continued the nicotine patch for another 16 weeks. By EOT (24 weeks), the standard group experienced a drop in quit rates (~10%) from week 8, resulting in significantly lower abstinence compared to extended treatment (20% vs 32%). However, by 28 weeks (only 1 month after EOT) the extended treatment group's abstinence also decreased by ~10%, and by 52 weeks abstinence was similar to that obtained with standard treatment (~14%).

These observations suggest that abstinence is maintained at ~30% as long as participants remain on nicotine patch, but once removed from nicotine patch abstinence falls to ~14% after 7 months. The high relapse rate during the 16-week placebo phase of the standard treatment group and after EOT (24 weeks) for both standard and extended treatment groups is likely due to the emergence of nicotine withdrawal and craving symptoms (Killen and Fortmann 1997). Furthermore, studies suggest that nicotine patch therapy reduces the risk of progression of an initial lapse to relapse (Shiffman *et al.* 2006a; Shiffman *et al.* 2006b), which would increase the odds of abstinence at EOT (24 weeks) for extended treatment and result in reduced abstinence once treatment is complete, as was observed in our study.

Contrary to expectations, extended nicotine patch treatment did not result in increased long-term abstinence. In fact, our findings were in agreement with a previous study that also did not find differences in long-term abstinence between 8-week and 22-week patch therapy (Tonnesen *et al.* 1999). Despite the findings by the latter study, the rationale for our hypothesis was that a longer duration of nicotine patch therapy would allow for more time to reduce the associations between nicotine and smoking behaviours, to adjust to the absence of behavioural aspects of smoking (e.g. holding a cigarette), to break associations of day-to-day behaviours with smoking (e.g. cigarette upon waking, cigarette at coffee break), and to learn how to control cravings and urges.

In addition, in our study population with an age of ~35 to 55 years and an age of smoking initiation of ~12 to 20 years, many smokers had been smoking for decades and up to 12 to 30 CPD. Perhaps smokers that fit this demographic require a treatment time longer than 6 months in order to successfully attain long-term abstinence. Future studies should explore the efficacy of longer patch treatment, e.g. 1 year or longer, or perhaps allow smokers to stay on nicotine patch therapy until they are ready to end treatment. Another possibility is to explore longer treatment with increased doses of nicotine. Although a meta-analysis suggests that there is minimal benefit from higher-dose nicotine patch (42/44mg vs 21/22mg nicotine via 24 hour patch, or 25mg vs 15mg nicotine via 16 hour patch) (Stead *et al.* 2008), perhaps a higher dose for longer duration would result in higher long-term abstinence rates.

4.4 Effect of 3HC/COT and CYP2A6 Genotype on Abstinence

We hypothesized that smokers with reduced CYP2A6 activity, defined both by low 3HC/COT and by slow *CYP2A6* genotype, would have increased quitting success compared to smokers with normal CYP2A6 activity. Our rationale was based on findings from previous

studies reporting that smokers with low 3HC/COT had higher quit rates with nicotine patch (Lerman *et al.* 2006), nicotine gum (Ho *et al.* 2009) and placebo treatment (Patterson *et al.* 2008; Ho *et al.* 2009) compared to those with high 3HC/COT. Since previous studies suggest that smokers with SM genotype may have an increased likelihood of quitting (Gu *et al.* 2000; Ho *et al.* 2009), we speculated that similar effects on abstinence could be obtained with *CYP2A6* genotype as with 3HC/COT.

Furthermore, smokers with low 3HC/COT or SM genotype smoke fewer CPD (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Benowitz *et al.* 2003; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006), and other studies have suggested smokers with SM genotype smoke for shorter durations and are less likely to be current smokers (Rao *et al.* 2000; Ariyoshi *et al.* 2002b; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Strasser *et al.* 2007). Perhaps the decreased intensity and duration of smoking by those with slow CYP2A6 activity could translate to more successful quitting. In addition, those with low 3HC/COT (Lerman *et al.* 2006) or those with SM genotype (Malaiyandi *et al.* 2006) obtained significantly higher levels of nicotine during nicotine patch therapy. We can speculate that perhaps increased nicotine levels from treatment leads to increased quit rates.

In our study, the analysis of baseline characteristics, treatment and smoking variables by *CYP2A6* genotype group suggested that our findings for the effect of low 3HC/COT on increasing abstinence could potentially be matched by an effect of SM genotype on increasing abstinence. For instance, *CYP2A6* genotype associated with 3HC/COT (Figure 3.3) and RMs obtained higher levels of nicotine during patch therapy compared to NMs (Figure 3.6). In addition, the RM group used for this analysis represented a similar proportion of the study population as Q1 (25%).

The first 8 weeks of nicotine patch treatment in our study was a replication of a previous study by Lerman *et al.* (2006) investigating the efficacy of 8-week patch therapy stratified by 3HC/COT quartile. In our study, after 8 weeks of treatment participants with low 3HC/COT (Q1) (treatment groups analyzed together) had higher quit rates compared to those with a high 3HC/COT (Q2-Q4) (42% vs 29%), which confirmed findings by Lerman *et al.* (2006). However, while consistent with our expectations, the difference in 8-week abstinence between RMs and NMs was not significantly different (38% vs 32%).

After week 8, abstinence was assessed within treatment groups. Q1 predicted higher abstinence within both treatment groups compared to Q2-Q4 (Figure 3.9 for extended treatment, Figure 3.10 for standard treatment), but abstinence was statistically higher only with extended treatment at week 8, EOT (24 weeks) and week 28. We expected to see a larger effect of 3HC/COT with standard therapy, since Lerman *et al*. (2006) previously showed that on standard 8-week therapy smokers with low 3HC/COT had higher quit rates at week 8 (EOT) and at 6-month follow-up compared to smokers with high 3HC/COT. It is unclear why Q1 had significantly higher quit rates than Q2-Q4 at week 8 of extended treatment, but not on standard treatment, given that standard and extended treatments were identical (21mg nicotine/day) until week 8. There were no differences in baseline characteristics between treatment groups that could explain this observation (Table 3.6). Therefore, the decreased likelihood of quitting by Q1 smokers placed on standard treatment could simply be an anomaly.

Contrary to expectations, our findings with *CYP2A6* genotype did not match our findings with 3HC/COT. There were no significant differences in abstinence at any of the time-points between RMs and NMs within the standard or extended treatment groups. On extended treatment, RMs had slightly higher quit rates at week 8 and EOT (24 weeks) compared to NMs,

but at follow-up RMs and NMs had similar quit rates (Figure 3.9). Within the standard treatment group, RMs had slightly higher abstinence at 8 weeks, but then demonstrated lower abstinence at EOT (24 weeks) and follow-up compared to NMs (Figure 3.10). Therefore, 3HC/COT was better associated with abstinence rates in both treatment groups compared to *CYP2A6* genotype, where low 3HC/COT predicted higher abstinence, particularly on extended treatment where differences were significant at 8 weeks, EOT (24 weeks) and at 1-month follow-up.

Despite our rationale for anticipating similar effects of 3HC/COT and *CYP2A6* genotype on abstinence, there are several explanations for the differences in predictability. For one, 3HC/COT is more representative of an individual's current nicotine metabolism compared to *CYP2A6* genotype. As discussed in section 4.1, there are many exogenous and endogenous influences on CYP2A6 enzymatic function, such as dietary compounds and medications, liver blood flow, gender and *CYP2A6* genetic variation. Using 3HC/COT as a measure of CYP2A6 activity takes into account much of the potential variability in CYP2A6 activity not captured by *CYP2A6* genotype. If 3HC/COT is a better indicator of CYP2A6 function compared to *CYP2A6* genotype, and if we expect that CYP2A6 function impacts quit rates with nicotine patch, then perhaps 3HC/COT is a better predictor of abstinence rates with nicotine patch compared to *CYP2A6* genotype.

The differences in the predictive ability of 3HC/COT versus *CYP2A6* genotype on abstinence may also be due to the fact that the 3HC/COT quartile groups were not equivalent to the *CYP2A6* genotype groups. For instance, the RM group was not identical to Q1 (55% of Q1 was RM, and 53% of RM was in Q1) and NM was not identical to Q2-Q4 (84% of Q2-Q4 was NM, and 85% of NM was in Q2-Q4). This was also reflected in the variability of 3HC/COT within each

genotype group (Figure 3.3), which suggested that although 3HC/COT associates with *CYP2A6* genotype, the two measures do not select identical individuals.

The limitations of genotyping, as discussed in section 4.1, also provide a reason for the differences in predictability of abstinence between 3HC/COT and genotype. There are several *CYP2A6* variants that we did not assay due to their very low frequency or uncharacterized impact on CYP2A6 function, and there may also be undiscovered *CYP2A6* variants. Since these variants were not detected with our genotyping assays, this may have led us to incorrectly categorize potential SMs or IMs as NMs. The fact that we did not genotype for *CYP2A6*1x2* and *CYP2A6*1B* variants is also a limitation, since individuals with these variants could perhaps be segregated into a fast metabolizer group, leading to a better characterization of CYP2A6 activity by genotype group and a larger effect of genotype on abstinence rates.

Comparisons were made between standard and extended treatment within each 3HC/COT and genotype group. We hypothesized that smokers in all 3HC/COT and genotype groups would achieve higher EOT (24 weeks) and long-term abstinence on extended treatment versus standard treatment, but that the largest benefit from extended treatment would be observed among those in Q2-Q4 or with NM genotype. The rationale for hypothesizing that smokers with high CYP2A6 activity would achieve a greater benefit from extended versus standard therapy is based on previous studies that suggest smokers with high 3HC/COT were less successful quitting on standard nicotine patch (Lerman *et al.* 2006), and that smokers with high 3HC/COT or NM genotype smoke more intensely and for longer durations (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Benowitz *et al.* 2003; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006; Strasser *et al.* 2007).

As expected, smokers in Q1 or with RM genotype achieved higher abstinence on extended therapy at EOT (24 weeks) and at 28 weeks compared to standard therapy (Figure 3.11), but the

benefits of longer treatment did not continue long after treatment ended. Contrary to expectations, however, smokers in Q2-Q4 and with the NM genotype only showed a slight improvement (not significant) in EOT (24 weeks) quit rates with extended versus standard treatment, and showed no differences at follow-up sessions (1 month or 7 months after EOT) (Figure 3.12). These findings were unexpected, given that we hypothesized all quartiles and genotype groups to have better quitting success on extended treatment at EOT (24 weeks), and furthermore that Q2-Q4 and NMs would show the largest gain in abstinence.

The different effects of treatment duration on Q1 versus Q2-4 and on RMs versus NMs provide future directions for nicotine patch treatment studies. Since smokers in Q1 or RMs responded advantageously to increased duration of patch therapy (i.e. as long as they remained on treatment), perhaps we should investigate even longer treatment (e.g. 1 year) for this subpopulation. If high abstinence can be maintained for an even longer duration, this may lead to increased odds of remaining abstinent long-term. On the other hand, smokers in Q2-4 or NMs did not benefit from longer treatment. Future trials should explore the effect of increasing the dose of nicotine patch (e.g. 42 mg/day nicotine with 24 hour patch) in this subpopulation, especially since the NMs in our study experienced lower nicotine levels from the patch therapy.

A future direction for this study in particular, is to create a model for abstinence rates within each treatment group, where we control for covariates such as gender, race, level of nicotine dependence or CPD, in order to assess the relationship between 3HC/COT, *CYP2A6* genotype and abstinence. The findings we reported here were raw quit rates, and perhaps controlling for covariates with a future model will allow for a more precise measure of the predictability of 3HC/COT and CYP2A6 genotype on abstinence.

4.5 Limitations of study

Although, the impact of *CYP2A6* genotype on nicotine patch efficacy compared to placebo has not been previously determined, this study did not include a control group (e.g. placebo patch for 24 weeks) because previous studies have shown that nicotine patch is effective compared to placebo. Therefore, the potential effect of *CYP2A6* genotype on quitting without active nicotine patch treatment in our study population was unknown. However, placebo-controlled clinical trials with nicotine gum (Ho *et al.* 2009) and bupropion (Patterson *et al.* 2008) suggest that SMs have higher quit rates on placebo treatment compared to NMs.

The exclusion criteria for our sample population in this study was extensive, and the final sample population most likely excluded several important groups present in the general smoking population, such as smokers who also suffer from other substance use disorders or psychiatric disorders. These co-morbidities could perhaps affect treatment outcomes with nicotine patch therapy, and instead of exclusion, perhaps some of the variables could have been included as covariates. For instance, there is a strong association between alcohol and tobacco use, where 80% of individuals dependent on alcohol also smoke cigarettes, and alcoholism is about 10x more common among smokers versus non-smokers (Romberger and Grant 2004). Therefore, alcohol dependence or history of alcohol use could have been included as a covariate, rather than excluding those who were alcohol dependent or with a history of use.

A history of major depression could have also been removed from the exclusion criteria. Although history of major depression does not appear to affect smoking cessation rates (meta-analysis: Hitsman *et al.* 2003), post-cessation major depression is more common in those with a previous history of major depression (Covey *et al.* 1997). It would have been interesting and potentially important to invest this population throughout treatment and follow them long-term.

Overall, incorporating several exclusion criteria as covariates or designing a study to include specialized populations would allowed us to explore whether extended nicotine patch treatment could increase quitting success in these groups, and whether 3HC/COT or *CYP2A6* genotype influence cessation.

The generalization of our findings regarding baseline characteristics (e.g. FTND, CPD, age of smoking initiation) to the general smoking population is limited by our exclusion criteria and because our study was designed for treatment-seeking smokers. Previous studies have shown that treatment-seeking smokers have different smoking behaviours and characteristics from those of the general smoking population (McGovern *et al.* 1994). For instance, compared to the general smoking population, smokers attending smoking cessation clinics were more likely to be women (although our study was made up of equal proportions of men and women), better educated, older, lighter in weight, white and married. They were also found to be heavier smokers.

Another limitation of the study was that only smokers that smoked >10 CPD were asked to participate, which excludes a population of light smokers that may also want to quit smoking. In this way, our study population was not representative of the smoking behaviours seen in the general population, but was consistent with most clinical trials. Furthermore, previous studies along with our present study have shown that smokers with slow CYP2A6 activity smoke fewer CPD (Rao *et al.* 2000; Ariyoshi *et al.* 2002a; Benowitz *et al.* 2003; Schoedel *et al.* 2004; Malaiyandi *et al.* 2006), which suggests that we are potentially excluding a population of light-smoking slow metabolizers from our study that are seeking cessation therapy. In addition, slow *CYP2A6* metabolizers smoke for shorter durations and are less likely to be adult smokers, and therefore they would be under-represented among long duration smokers (Schoedel *et al.* 2004). It may be that treatment-seeking smokers with slow CYP2A6 activity represent the portion of

slow metabolizers that have previously failed cessation therapy and/or have had a difficult time quitting on their own. This under-representation of slow metabolizers in cessation trials could reduce the effect of *CYP2A6* genotype on abstinence on the total smoking population.

There are also limitations inherent in genotyping and the assays that we chose to perform. As mentioned, we did not genotype for all known slow *CYP2A6* variants, as well as some variants that increase CYP2A6 activity, such as *CYP2A6*1x2* and *CYP2A6*1B*. There may also be unknown *CYP2A6* variants that were not detected by our genotyping assays. This means that some individuals were incorrectly categorized into a genotype group, which would reduce the power of detecting the effect of *CYP2A6* on quitting rates with nicotine patch.

5. GENERAL CONCLUSIONS

Aim #1: Efficacy of Standard versus Extended Nicotine Patch Therapy

1. Maintenance on nicotine patch therapy (extended treatment) resulted in maintenance of abstinence rates.
2. Extended treatment did not increase long-term abstinence.

Aim #2: Effect of *CYP2A6* Genotype on Efficacy of Nicotine Patch Therapy

3. RM genotype trended towards increased abstinence on *extended* treatment at 8 weeks and EOT (24 weeks) compared to NM genotype, but the differences did not reach significance. Genotype did not predict long-term abstinence (52 weeks).
4. RM genotype predicted higher abstinence with extended treatment compared to standard treatment at EOT (24 weeks). Extended treatment did not increase long-term abstinence (52 weeks) for those with RM genotype.
5. NM genotype did not predict a difference in abstinence between standard and extended patch therapy at EOT (24 weeks) or long-term (52 weeks).

Aim #3: Comparison of *CYP2A6* Genotype and 3HC/COT as Predictors of Smoking Abstinence

6. Unlike RM genotype (summarized in **conclusion 3**), low 3HC/COT predicted higher abstinence on *extended* treatment compared to high 3HC/COT at 8 weeks, EOT (24 weeks) and 28 weeks. Similar to RM genotype, 3HC/COT did not predict long-term abstinence (52 weeks).
7. Similar to RM genotype (summarized in **conclusion 4**), low 3HC/COT predicted higher abstinence with extended treatment compared to standard treatment at EOT (24 weeks) and 28 weeks. Extended treatment did not increase long-terms abstinence (52 weeks) for those with low 3HC/COT.

8. Similar to NM genotype (summarized in **conclusion 5**), high 3HC/COT did not predict a difference in abstinence between standard and extended patch therapy at EOT (24 weeks) or long-term (52 weeks).

Additional Conclusions for Smoking and Treatment Variables:

9. *CYP2A6* genotype associated with baseline CPD, where SMs smoked fewer CPD compared to NMs.
10. *CYP2A6* genotype associated with baseline 3HC/COT, where SMs had lower 3HC/COT and thus lower CYP2A6 activity compared to NMs.
11. *CYP2A6* genotype associated with plasma nicotine and COT levels obtained from patch treatment, where SMs obtained higher plasma nicotine and COT levels compared to NMs.

6. REFERENCES

Al Koudsi N, Ahluwalia JS, Lin SK, Sellers EM, Tyndale RF (2009) A novel CYP2A6 allele (CYP2A6(*)35) resulting in an amino-acid substitution (Asn438Tyr) is associated with lower CYP2A6 activity in vivo. Pharmacogenomics J

Al Koudsi N, Mwenifumbo JC, Sellers EM, Benowitz NL, Swan GE, Tyndale RF (2006) Characterization of the novel CYP2A6*21 allele using in vivo nicotine kinetics. Eur J Clin Pharmacol 62: 481-4

Ariyoshi N, Miyamoto M, Umetsu Y, Kunitoh H, Dosaka-Akita H, Sawamura Y, Yokota J, Nemoto N, Sato K, Kamataki T (2002a) Genetic polymorphism of CYP2A6 gene and tobacco-induced lung cancer risk in male smokers. Cancer Epidemiol Biomarkers Prev 11: 890-4

Ariyoshi N, Sawamura Y, Kamataki T (2001) A novel single nucleotide polymorphism altering stability and activity of CYP2a6. Biochem Biophys Res Commun 281: 810-4

Ariyoshi N, Sekine H, Nakayama K, Saito K, Miyamoto A, Kamataki T (2004) Identification of deletion-junction site of CYP2A6*4B allele lacking entire coding region of CYP2A6 in Japanese. Pharmacogenetics 14: 701-5

Ariyoshi N, Sekine H, Saito K, Kamataki T (2002b) Characterization of a genotype previously designated as CYP2A6 D-type: CYP2A6*4B, another entire gene deletion allele of the CYP2A6 gene in Japanese. Pharmacogenetics 12: 501-4

Ariyoshi N, Takahashi Y, Miyamoto M, Umetsu Y, Daigo S, Tateishi T, Kobayashi S, Mizorogi Y, Loriot MA, Stucker I, Beaune P, Kinoshita M, Kamataki T (2000) Structural characterization of a new variant of the CYP2A6 gene (CYP2A6*1B) apparently diagnosed as heterozygotes of CYP2A6*1A and CYP2A6*4C. Pharmacogenetics 10: 687-93

Audrain-McGovern J, Al Koudsi N, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF (2007) The role of CYP2A6 in the emergence of nicotine dependence in adolescents. Pediatrics 119: e264-74

Benowitz N, Tyndale R, Jacob P, 3rd, Swan GE (2002a) CYP2A6 Polymorphism and Nicotine Metabolism. Clinical Pharmacology and Therapeutics 71: P41

Benowitz NL (1996) Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol 36: 597-613

Benowitz NL (2008) Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther 83: 531-41

Benowitz NL, Jacob P, 3rd (1985) Nicotine renal excretion rate influences nicotine intake during cigarette smoking. J Pharmacol Exp Ther 234: 153-5

Benowitz NL, Jacob P, 3rd (1994) Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther 56: 483-93

Benowitz NL, Jacob P, 3rd (2001) Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. Br J Clin Pharmacol 51: 53-9

Benowitz NL, Jacob P, 3rd, Fong I, Gupta S (1994) Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. J Pharmacol Exp Ther 268: 296-303

Benowitz NL, Jacob P, 3rd, Jones RT, Rosenberg J (1982) Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther 221: 368-72

Benowitz NL, Jacob P, 3rd, Sachs DP (1995) Deficient C-oxidation of nicotine. Clin Pharmacol Ther 57: 590-4
Benowitz NL, Kuyt F, Jacob P, 3rd, Jones RT, Osman AL (1983) Cotinine disposition and effects. Clin Pharmacol Ther 34: 604-11
Benowitz NL, Lessov-Schlaggar CN, Swan GE, Jacob P, 3rd (2006a) Female sex and oral contraceptive use accelerate nicotine metabolism. Clin Pharmacol Ther 79: 480-8
Benowitz NL, Perez-Stable EJ, Fong I, Modin G, Herrera B, Jacob P, 3rd (1999) Ethnic differences in N-glucuronidation of nicotine and cotinine. J Pharmacol Exp Ther 291: 1196-203
Benowitz NL, Perez-Stable EJ, Herrera B, Jacob P, 3rd (2002b) Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. J Natl Cancer Inst 94: 108-15
Benowitz NL, Pomerleau OF, Pomerleau CS, Jacob P, 3rd (2003) Nicotine metabolite ratio as a predictor of cigarette consumption. Nicotine Tob Res 5: 621-4
Benowitz NL, Swan GE, Jacob P, 3rd, Lessov-Schlaggar CN, Tyndale RF (2006b) CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. Clin Pharmacol Ther 80: 457-67
Benowitz NL, Zevin S, Jacob P, 3rd (1997) Sources of variability in nicotine and cotinine levels with use of nicotine nasal spray, transdermal nicotine, and cigarette smoking. Br J Clin Pharmacol 43: 259-67
Biovail Pharmaceuticals Canada (2005) Zyban (Bupropion Hydrochloride) 150mg Sustained Release Tablets: Smoking Cessation Aid
Bolin LJ, Antonuccio DO, Follette WC, Krumpe P (1999) Transdermal nicotine: the long and the short of it. Psychology of Addictive Behaviors 13: 152-6
Boshier A, Wilton LV, Shakir SA (2003) Evaluation of the safety of bupropion (Zyban) for smoking cessation from experience gained in general practice use in England in 2000. Eur J Clin Pharmacol 59: 767-73
Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J (2006) Genetic architecture of smoking behavior: a study of Finnish adult twins. Twin Res Hum Genet 9: 64-72
Buisson B, Bertrand D (2002) Nicotine addiction: the possible role of functional upregulation. Trends Pharmacol Sci 23: 130-6
Carmelli D, Swan GE, Robinette D, Fabsitz R (1992) Genetic influence on smoking--a study of male twins. N Engl J Med 327: 829-33
CDC (2002) Cigarette smoking among adults--United States, 2000. MMWR Morb Mortal Wkly Rep 51: 642-5
CDC (2004) 2004 Surgeon General's Report - The Health Consequences of Smoking. Department of Health and Human Services: Centers for Disease Control and Prevention.
CDC (2005) Cigarette smoking among adults--United States, 2004. MMWR Morb Mortal Wkly Rep 54: 1121-4
Cepeda-Benito A, Reynoso JT, Erath S (2004) Meta-analysis of the efficacy of nicotine replacement therapy for smoking cessation: differences between men and women. J Consult Clin Psychol 72: 712-22
Cholerton S, Arpanahi A, McCracken N, Boustead C, Taber H, Johnstone E, Leathart J, Daly AK, Idle JR (1994) Poor metabolisers of nicotine and CYP2D6 polymorphism. Lancet 343: 62-3

Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollema H, Lu Y, Mansbach RS, Chambers LK, Rovetti CC, Schulz DW, Tingley FD, 3rd, O'Neill BT (2005) Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. J Med Chem 48: 3474-7

Corrigall WA, Coen KM (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl) 99: 473-8

Covey LS, Glassman AH, Stetner F (1997) Major depression following smoking cessation. Am J Psychiatry 154: 263-265

Cox LS, Patten CA, Niaura RS, Decker PA, Rigotti N, Sachs DP, Buist AS, Hurt RD (2004) Efficacy of bupropion for relapse prevention in smokers with and without a past history of major depression. J Gen Intern Med 19: 828-34

Cryan JF, Bruijnzeel AW, Skjei KL, Markou A (2003) Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. Psychopharmacology (Berl) 168: 347-58

CTUMS (2008) Canadian Tobacco Use Monitoring Survery (CTUMS) 2008, Ottawa: Health Canada

Daigo S, Takahashi Y, Fujieda M, Ariyoshi N, Yamazaki H, Koizumi W, Tanabe S, Saigenji K, Nagayama S, Ikeda K, Nishioka Y, Kamataki T (2002) A novel mutant allele of the CYP2A6 gene (CYP2A6*11) found in a cancer patient who showed poor metabolic phenotype towards tegafur. Pharmacogenetics 12: 299-306

Dani JA, Heinemann S (1996) Molecular and cellular aspects of nicotine abuse. Neuron 16: 905-8

Dempsey D, Tutka P, Jacob P, 3rd, Allen F, Schoedel K, Tyndale RF, Benowitz NL (2004) Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 76: 64-72

Dempsey DA, Benowitz NL (2001) Risks and benefits of nicotine to aid smoking cessation in pregnancy. Drug Saf 24: 277-322

Denton TT, Zhang X, Cashman JR (2004) Nicotine-related alkaloids and metabolites as inhibitors of human cytochrome P-450 2A6. Biochem Pharmacol 67: 751-6

Ding S, Lake BG, Friedberg T, Wolf CR (1995) Expression and alternative splicing of the cytochrome P-450 CYP2A7. Biochem J 306 (Pt 1): 161-6

Djordjevic MV, Fan J, Ferguson S, Hoffmann D (1995) Self-regulation of smoking intensity. Smoke yields of the low-nicotine, low-'tar' cigarettes. Carcinogenesis 16: 2015-21

Dome P, Lazary J, Kalapos MP, Rihmer Z (2009) Smoking, nicotine and neuropsychiatric disorders. Neurosci Behav Rev doi:10.1016/j.neubiorev.2009.07.013

Dunner DL, Zisook S, Billow AA, Batey SR, Johnston JA, Ascher JA (1998) A prospective safety surveillance study for bupropion sustained-release in the treatment of depression. J Clin Psychiatry 59: 366-73

Ebbert JO, Post JA, Moyer TP, Dale LC, Schroeder DR, Hurt RD (2007) Nicotine percentage replacement among smokeless tobacco users with nicotine patch. Drug Alcohol Depend 89: 223-226

Ekins S, Wrighton SA (1999) The role of CYP2B6 in human xenobiotic metabolism. Drug Metab Rev 31: 719-54

Elkins JG, Hassett DJ, Stewart PS, Schweizer HP, McDermott TR (1999) Protective role of catalase in Pseudomonas aeruginosa biofilm resistance to hydrogen peroxide. Appl Environ Microbiol 65: 4594-600
Elkins TJ, Phillips JS (1999) Evaluating sex discrimination claims: the mediating role of attributions. J Appl Psychol 84: 186-99
Epping-Jordan MP, Watkins SS, Koob GF, Markou A (1998) Dramatic decreases in brain reward function during nicotine withdrawal. Nature 393: 76-9
Etter JF (2008) Comparing the validity of the Cigarette Dependence Scale and the Fagerstrom Test for Nicotine Dependence. Drug Alcohol Depend 95: 152-9
Fagerstrom KO, Heatherton TF, Kozlowski LT (1990a) Nicotine addiction and its assessment. Ear Nose Throat J 69: 763-5
Fenster CP, Whitworth TL, Sheffield EB, Quick MW, Lester RA (1999) Upregulation of surface alpha4beta2 nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. J Neurosci 19: 4804-14
Fernandez-Salguero P, Hoffman SM, Cholerton S, Mohrenweiser H, Raunio H, Rautio A, Pelkonen O, Huang JD, Evans WE, Idle JR, et al. (1995) A genetic polymorphism in coumarin 7-hydroxylation: sequence of the human CYP2A genes and identification of variant CYP2A6 alleles. Am J Hum Genet 57: 651-60
Fiore MC, Jaen CR, Baker TB, Bailey WC, Benowitz N, Curry SJ (2008) Treating tobacco use and dependence: 2008 Update Clinical Practice Guideline. U.S. Department of Health and Human Services, Public Health Service, Rockville, MD.
Fryer JD, Lukas RJ (1999) Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. J Pharmacol Exp Ther 288: 88-92
Fukami T, Nakajima M, Higashi E, Yamanaka H, McLeod HL, Yokoi T (2005a) A novel CYP2A6*20 allele found in African-American population produces a truncated protein lacking enzymatic activity. Biochem Pharmacol 70: 801-8
Fukami T, Nakajima M, Higashi E, Yamanaka H, Sakai H, McLeod HL, Yokoi T (2005b) Characterization of novel CYP2A6 polymorphic alleles (CYP2A6*18 and CYP2A6*19) that affect enzymatic activity. Drug Metab Dispos 33: 1202-10
Fukami T, Nakajima M, Yamanaka H, Fukushima Y, McLeod HL, Yokoi T (2007) A novel duplication type of CYP2A6 gene in African-American population. Drug Metab Dispos 35: 515-20
Fukami T, Nakajima M, Yoshida R, Tsuchiya Y, Fujiki Y, Katoh M, McLeod HL, Yokoi T (2004) A novel polymorphism of human CYP2A6 gene CYP2A6*17 has an amino acid substitution (V365M) that decreases enzymatic activity in vitro and in vivo. Clin Pharmacol Ther 76: 519-27
Glavas D, Rumboldt Z (2003) [Smoking cessation using the transdermal nicotine system]. Lijec Vjesn 125: 8-12
Gonzales D, Bjornson W, Durcan MJ, White JD, Johnston JA, Buist AS, Sachs DP, Rigotti NA, Niaura R, Hays JT, Hurt RD (2002) Effects of gender on relapse prevention in smokers treated with bupropion SR. Am J Prev Med 22: 234-9
Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, Watsky EJ, Gong J, Williams KE, Reeves KR (2006) Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 296: 47-55

Gonzales DH, Nides MA, Ferry LH, Kustra RP, Jamerson BD, Segall N, Herrero LA, Krishen A, Sweeney A, Buaron K, Metz A (2001) Bupropion SR as an aid to smoking cessation in smokers treated previously with bupropion: a randomized placebo-controlled study. Clin Pharmacol Ther 69: 438-44

Gu DF, Hinks LJ, Morton NE, Day IN (2000) The use of long PCR to confirm three common alleles at the CYP2A6 locus and the relationship between genotype and smoking habit. Ann Hum Genet 64: 383-90

Haberl M, Anwald B, Klein K, Weil R, Fuss C, Gepdiremen A, Zanger UM, Meyer UA, Wojnowski L (2005) Three haplotypes associated with CYP2A6 phenotypes in Caucasians. Pharmacogenet Genomics 15: 609-24

Hadidi H, Zahlsen K, Idle JR, Cholerton S (1997) A single amino acid substitution (Leu160His) in cytochrome P450 CYP2A6 causes switching from 7-hydroxylation to 3-hydroxylation of coumarin. Food Chem Toxicol 35: 903-7

Hayford KE, Patten CA, Rummans TA, Schroeder DR, Offord KP, Croghan IT, Glover ED, Sachs DP, Hurt RD (1999) Efficacy of bupropion for smoking cessation in smokers with a former history of major depression or alcoholism. Br J Psychiatry 174: 173-8

Heath AC, Kirk KM, Meyer JM, Martin NG (1999) Genetic and social determinants of initiation and age at onset of smoking in Australian twins. Behav Genet 29: 395-407

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 86: 1119-27

Henningfield JE, Miyasato K, Jasinski DR (1985) Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. J Pharmacol Exp Ther 234: 1-12

Higashi E, Fukami T, Itoh M, Kyo S, Inoue M, Yokoi T, Nakajima M (2007) Human CYP2A6 is induced by estrogen via estrogen receptor. Drug Metab Dispos 35: 1935-41

Hitsman B, Borelli B, McCharque DE, Spring B, Niaura R (2003) History of depression and smoking cessation outcome: a meta-analysis. J Consult Clin Psychol 71: 657-663

Ho M, Mwenifumbo J, Al Koudsi N, Okuyemi K, Ahluwalia J, Benowitz N, Tyndale R (2009) Association of Nicotine Metabolite Ratio and CYP2A6 Genotype With Smoking Cessation Treatment in African-American Light Smokers. Clin Pharmacol Ther

Ho MK, Mwenifumbo JC, Zhao B, Gillam EM, Tyndale RF (2008) A novel CYP2A6 allele, CYP2A6*23, impairs enzyme function in vitro and in vivo and decreases smoking in a population of Black-African descent. Pharmacogenet Genomics 18: 67-75

Ho MK, Tyndale RF (2007) Overview of the pharmacogenomics of cigarette smoking. Pharmacogenomics J 7: 81-98

Hoffman SM, Fernandez-Salguero P, Gonzalez FJ, Mohrenweiser HW (1995) Organization and evolution of the cytochrome P450 CYP2A-2B-2F subfamily gene cluster on human chromosome 19. J Mol Evol 41: 894-900

Hoffman SM, Nelson DR, Keeney DS (2001) Organization, structure and evolution of the CYP2 gene cluster on human chromosome 19. Pharmacogenetics 11: 687-98

Hubbard R, Lewis S, Smith C, Godfrey C, Smeeth L, Farrington P, Britton J (2005) Use of nicotine replacement therapy and the risk of acute myocardial infarction, stroke, and death. Tob Control 14: 416-21

Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW (1991) Symptoms of tobacco withdrawal. A replication and extension. Arch Gen Psychiatry 48: 52-9

Hughes JR, Hatsukami D (1986) Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry 43: 289-94
Hughes JR, Keely J, Naud S (2004) Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction 99: 29-38
Hughes JR, Stead LF, Lancaster T (2007) Antidepressants for smoking cessation. Cochrane Database Syst Rev: CD000031
Hukkanen J, Jacob P, 3rd, Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev 57: 79-115
Hurt RD, Wolter TD, Rigotti N, Hays JT, Niaura R, Durcan MJ, Gonzales D, Sachs DP, Johnston JA, Offord KP (2002) Bupropion for pharmacologic relapse prevention to smoking: predictors of outcome. Addict Behav 27: 493-507
Ikeda K, Yoshisue K, Matsushima E, Nagayama S, Kobayashi K, Tyson CA, Chiba K, Kawaguchi Y (2000) Bioactivation of tegafur to 5-fluorouracil is catalyzed by cytochrome P-450 2A6 in human liver microsomes in vitro. Clin Cancer Res 6: 4409-15
Imura K, Ueda Y, Hayashi T, Itoh T, Shimizu K, Tamai H, Yano Y, Naito K, Kohara J, Nakane K, Matsuura Y, Takeda A, Takeda T, Kawai K, Yamagishi H (2006) Induction of cytotoxic T lymphocytes against human cancer cell lines using dendritic cell-tumor cell hybrids generated by a newly developed electrofusion technique. Int J Oncol 29: 531-9
Ingram RE, Busch W, Christianson-Strom C, Fagerstrom C, Hale M, Himes P, Kovak K, Marymee K (1990) What our patients are reading about reproductive technologies. A review of eight popular women's magazines, 1978 and 1988. Birth Defects Orig Artic Ser 26: 105-8
Itoh M, Nakajima M, Higashi E, Yoshida R, Nagata K, Yamazoe Y, Yokoi T (2006) Induction of human CYP2A6 is mediated by the pregnane X receptor with peroxisome proliferator-activated receptor-gamma coactivator 1alpha. J Pharmacol Exp Ther 319: 693-702
Iwahashi K, Waga C, Takimoto T (2004) Whole deletion of CYP2A6 gene (CYP2A6AST;4C) and smoking behavior. Neuropsychobiology 49: 101-4
Jacob P, 3rd, Wilson M, Benowitz NL (1981) Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. J Chromatogr 222: 61-70
Jarvik ME, Madsen DC, Olmstead RE, Iwamoto-Schaap PN, Elins JL, Benowitz NL (2000) Nicotine blood levels and subjective craving for cigarettes. Pharmacol Biochem Behav 66: 553-8
Jarvis MJ (2004) Why people smoke. BMJ 328: 277-9
Jorenby D (2002) Clinical efficacy of bupropion in the management of smoking cessation. Drugs 62 Suppl 2: 25-35
Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR (2006) Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA 296: 56-63
Joseph AM, Norman SM, Ferry LH, Prochazka AV, Westman EC, Steele BG, Sherman SE, Cleveland M, Antonuccio DO, Hartman N, McGovern PG (1996) The safety of transdermal nicotine as an aid to smoking cessation in patients with cardiac disease. N Engl J Med 335: 1792-8
Kandel DB, Chen K (2000) Extent of smoking and nicotine dependence in the United States: 1992-1993. Nicotine Tob Res 2: 263-274

Kassel JD, Greenstein JE, Evatt DP, Wardle MC, Yates MC, Veilleux JC, Eissenberg T (2007) Smoking topography in response to denicotinized and high-yield nicotine cigarettes in adolescent smokers. J Adolesc Health 40: 54-60

Kawakami N, Takatsuka N, Inaba S, Shimizu H (1999) Development of a screening questionnaire for tobacco/nicotine dependence according to ICD-10, DSM-III-R, and DSM-IV. Addict Behav 24: 155-66

Kendler KS, Thornton LM, Pedersen NL (2000) Tobacco consumption in Swedish twins reared apart and reared together. Arch Gen Psychiatry 57: 886-92

Kessler RC, Adler L, Ames M, Demler O, Faraone S, Hiripi E, Howes MJ, Jin R, Secnik K, Spencer T, Ustun TB, Walters EE (2005) The World Health Organization Adult ADHD Self-Report Scale (ASRS): a short screening scale for use in the general population. Psychol Med 35: 245-56

Kharasch ED, Hankins DC, Taraday JK (2000) Single-dose methoxsalen effects on human cytochrome P-450 2A6 activity. Drug Metab Dispos 28: 28-33

Kharasch ED, Hankins DC, Thummel KE (1995) Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. Anesthesiology 82: 689-99

Killen JD, Fortmann SP (1997) Craving is associated with smoking relapse: findings from three prospective studies. Exp Clin Psychopharmacol 5: 137-42

Kiyotani K, Yamazaki H, Fujieda M, Iwano S, Matsumura K, Satarug S, Ujjin P, Shimada T, Guengerich FP, Parkinson A, Honda G, Nakagawa K, Ishizaki T, Kamataki T (2003) Decreased coumarin 7-hydroxylase activities and CYP2A6 expression levels in humans caused by genetic polymorphism in CYP2A6 promoter region (CYP2A6*9). Pharmacogenetics 13: 689-95

Komatsu T, Yamazaki H, Shimada N, Nakajima M, Yokoi T (2000) Roles of cytochromes P450 1A2, 2A6, and 2C8 in 5-fluorouracil formation from tegafur, an anticancer prodrug, in human liver microsomes. Drug Metab Dispos 28: 1457-63

Koopmans JR, Slutske WS, Heath AC, Neale MC, Boomsma DI (1999) The genetics of smoking initiation and quantity smoked in Dutch adolescent and young adult twins. Behav Genet 29: 383-93

Koskela S, Hakkola J, Hukkanen J, Pelkonen O, Sorri M, Saranen A, Anttila S, Fernandez-Salguero P, Gonzalez F, Raunio H (1999) Expression of CYP2A genes in human liver and extrahepatic tissues. Biochem Pharmacol 57: 1407-13

Kuehl GE, Murphy SE (2003) N-glucuronidation of nicotine and cotinine by human liver microsomes and heterologously expressed UDP-glucuronosyltransferases. Drug Metab Dispos 31: 1361-8

Kwon JT, Nakajima M, Chai S, Yom YK, Kim HK, Yamazaki H, Sohn DR, Yamamoto T, Kuroiwa Y, Yokoi T (2001) Nicotine metabolism and CYP2A6 allele frequencies in Koreans. Pharmacogenetics 11: 317-23

Lang DH, Yeung CK, Peter RM, Ibarra C, Gasser R, Itagaki K, Philpot RM, Rettie AE (1998) Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. Biochem Pharmacol 56: 1005-12

Laviolette SR, van der Kooy D (2003) Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. Mol Psychiatry 8: 50-9, 9

Le Foll B, George TP (2007) Treatment of tobacco dependence: integrating recent progress into practice. CMAJ 177: 1373-80
Le Foll B, Goldberg SR (2005) Nicotine induces conditioned place preferences over a large range of doses in rats. Psychopharmacology (Berl) 178: 481-92
Le Foll B, Goldberg SR (2006) Nicotine as a typical drug of abuse in experimental animals and humans. Psychopharmacology (Berl) 184: 367-81
Le Foll B, Wertheim C, Goldberg SR (2007) High reinforcing efficacy of nicotine in non-human primates. PLoS ONE 2: e230
Le Foll B, Wiggins M, Goldberg SR (2006) Nicotine pre-exposure does not potentiate the locomotor or rewarding effects of Delta-9-tetrahydrocannabinol in rats. Behav Pharmacol 17: 195-9
Lerman C, Kaufmann V, Rukstalis M, Patterson F, Perkins K, Audrain-McGovern J, Benowitz N (2004) Individualizing nicotine replacement therapy for the treatment of tobacco dependence: a randomized trial. Ann Intern Med 140: 426-33
Lerman C, Roth D, Kaufmann V, Audrain J, Hawk L, Liu A, Niaura R, Epstein L (2002) Mediating mechanisms for the impact of bupropion in smoking cessation treatment. Drug Alcohol Depend 67: 219-23
Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, Benowitz N (2006) Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther 79: 600-8
Lerman CE, Schnoll RA, Munafo MR (2007) Genetics and smoking cessation improving outcomes in smokers at risk. Am J Prev Med 33: S398-405
Lessov CN, Martin NG, Statham DJ, Todorov AA, Slutske WS, Bucholz KK, Heath AC, Madden PA (2004) Defining nicotine dependence for genetic research: evidence from Australian twins. Psychol Med 34: 865-79
Li MD, Cheng R, Ma JZ, Swan GE (2003) A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction 98: 23-31
Lockman PR, McAfee G, Geldenhuys WJ, Van der Schyf CJ, Abbruscato TJ, Allen DD (2005) Brain uptake kinetics of nicotine and cotinine after chronic nicotine exposure. J Pharmacol Exp Ther 314: 636-42
Loriot MA, Rebuissou S, Oscarson M, Cenee S, Miyamoto M, Ariyoshi N, Kamataki T, Hemon D, Beaune P, Stucker I (2001) Genetic polymorphisms of cytochrome P450 2A6 in a case-control study on lung cancer in a French population. Pharmacogenetics 11: 39-44
Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F, Tyndale RF (2006) Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. Mol Psychiatry 11: 400-9
Malaiyandi V, Sellers EM, Tyndale RF (2005) Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. Clin Pharmacol Ther 77: 145-58
Marks MJ, Burch JB, Collins AC (1983) Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J Pharmacol Exp Ther 226: 817-25
McGovern PG, Lando HA, Roski J, Pirie PL, Sprafka JM (1994) A comparison of smoking cessation clinic participants with smokers in the general population. Tob Control 3: 329-333
McGue M, Elkins I, Iacono WG (2000) Genetic and environmental influences on adolescent substance use and abuse. Am J Med Genet 96: 671-7

McMorrow MJ, Foxx RM (1983) Nicotine's role in smoking: an analysis of nicotine regulation. Psychol Bull 93: 302-27
McNeil Consumer Healthcare (2008) Product Monograph: Nicorette Patch.
McRobbie H, Hajek P (2001) Nicotine replacement therapy in patients with cardiovascular disease: guidelines for health professionals. Addiction 96: 1547-51
Messina ES, Tyndale RF, Sellers EM (1997) A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 282: 1608-14
Miles JS, McLaren AW, Forrester LM, Glancey MJ, Lang MA, Wolf CR (1990) Identification of the human liver cytochrome P-450 responsible for coumarin 7-hydroxylase activity. Biochem J 267: 365-71
Moritsugu KP (2007) The 2006 Report of the Surgeon General: the health consequences of involuntary exposure to tobacco smoke. Am J Prev Med 32: 542-3
Munafo M (2004) Replication validity of genetic association studies of smoking behavior: what can meta-analytic techniques offer? Nicotine Tob Res 6: 381-2
Munafo M, Bradburn M, Bowes L, David S (2004a) Are there sex differences in transdermal nicotine replacement therapy patch efficacy? A meta-analysis. Nicotine Tob Res 6: 769-76
Munafo M, Clark T, Johnstone E, Murphy M, Walton R (2004b) The genetic basis for smoking behavior: a systematic review and meta-analysis. Nicotine Tob Res 6: 583-97
Murphy SE, Johnson LM, Pullo DA (1999) Characterization of multiple products of cytochrome P450 2A6-catalyzed cotinine metabolism. Chem Res Toxicol 12: 639-45
Mwenifumbo JC, Al Koudsi N, Ho MK, Zhou Q, Hoffmann EB, Sellers EM, Tyndale RF (2008) Novel and established CYP2A6 alleles impair in vivo nicotine metabolism in a population of Black African descent. Hum Mutat 29: 679-88
Mwenifumbo JC, Myers MG, Wall TL, Lin SK, Sellers EM, Tyndale RF (2005) Ethnic variation in CYP2A6*7, CYP2A6*8 and CYP2A6*10 as assessed with a novel haplotyping method. Pharmacogenet Genomics 15: 189-92
Mwenifumbo JC, Tyndale RF (2007) Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. Pharmacogenomics 8: 1385-402
Nakajima M, Fukami T, Yamanaka H, Higashi E, Sakai H, Yoshida R, Kwon JT, McLeod HL, Yokoi T (2006) Comprehensive evaluation of variability in nicotine metabolism and CYP2A6 polymorphic alleles in four ethnic populations. Clin Pharmacol Ther 80: 282-97
Nakajima M, Kwon JT, Tanaka N, Zenta T, Yamamoto Y, Yamamoto H, Yamazaki H, Yamamoto T, Kuroiwa Y, Yokoi T (2001) Relationship between interindividual differences in nicotine metabolism and CYP2A6 genetic polymorphism in humans. Clin Pharmacol Ther 69: 72-8
Nakajima M, Yamagishi S, Yamamoto H, Yamamoto T, Kuroiwa Y, Yokoi T (2000) Deficient cotinine formation from nicotine is attributed to the whole deletion of the CYP2A6 gene in humans. Clin Pharmacol Ther 67: 57-69
Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, Kuroiwa Y (1996a) Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. J Pharmacol Exp Ther 277: 1010-5
Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, Kuroiwa Y (1996b) Role of human cytochrome P4502A6 in C-oxidation of nicotine. Drug Metab Dispos 24: 1212-7

Nakajima M, Yokoi T (2005) Interindividual variability in nicotine metabolism: C-oxidation and glucuronidation. Drug Metab Pharmacokinet 20: 227-35

Nakamura M, Oshima A, Fujimoto Y, Maruyama N, Ishibashi T, Reeves KR (2007) Efficacy and tolerability of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, in a 12-week, randomized, placebo-controlled, dose-response study with 40-week follow-up for smoking cessation in Japanese smokers. Clin Ther 29: 1040-56

Neurath GB (1994) Aspects of the oxidative metabolism of nicotine. Clin Investig 72: 190-5

Nides M (2008) Update on pharmacologic options for smoking cessation treatment. Am J Med 121: S20-31

Nides M, Oncken C, Gonzales D, Rennard S, Watsky EJ, Anziano R, Reeves KR (2006) Smoking cessation with varenicline, a selective alpha4beta2 nicotinic receptor partial agonist: results from a 7-week, randomized, placebo- and bupropion-controlled trial with 1-year follow-up. Arch Intern Med 166: 1561-8

Nunoya K, Yokoi T, Kimura K, Inoue K, Kodama T, Funayama M, Nagashima K, Funae Y, Green C, Kinoshita M, Kamataki T (1998) A new deleted allele in the human cytochrome P450 2A6 (CYP2A6) gene found in individuals showing poor metabolic capacity to coumarin and (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride (SM-12502). Pharmacogenetics 8: 239-49

Nunoya K, Yokoi T, Takahashi Y, Kimura K, Kinoshita M, Kamataki T (1999a) Homologous unequal cross-over within the human CYP2A gene cluster as a mechanism for the deletion of the entire CYP2A6 gene associated with the poor metabolizer phenotype. J Biochem 126: 402-7

Nunoya K, Yokoi Y, Kimura K, Kodama T, Funayama M, Inoue K, Nagashima K, Funae Y, Shimada N, Green C, Kamataki T (1996) (+)-cis-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride (SM-12502) as a novel substrate for cytochrome P450 2A6 in human liver microsomes. J Pharmacol Exp Ther 277: 768-74

Nunoya KI, Yokoi T, Kimura K, Kainuma T, Satoh K, Kinoshita M, Kamataki T (1999b) A new CYP2A6 gene deletion responsible for the in vivo polymorphic metabolism of (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride in humans. J Pharmacol Exp Ther 289: 437-42

O'Loughlin J, Paradis G, Kim W, DiFranza J, Meshefedjian G, McMillan-Davey E, Wong S, Hanley J, Tyndale RF (2004) Genetically decreased CYP2A6 and the risk of tobacco dependence: a prospective study of novice smokers. Tob Control 13: 422-8

Oncken C, Gonzales D, Nides M, Rennard S, Watsky E, Billing CB, Anziano R, Reeves K (2006) Efficacy and safety of the novel selective nicotinic acetylcholine receptor partial agonist, varenicline, for smoking cessation. Arch Intern Med 166: 1571-7

Onica T, Nichols K, Larin M, Ng L, Maslen A, Dvorak Z, Pascussi JM, Vilarem MJ, Maurel P, Kirby GM (2008) Dexamethasone-mediated up-regulation of human CYP2A6 involves the glucocorticoid receptor and increased binding of hepatic nuclear factor 4 alpha to the proximal promoter. Mol Pharmacol 73: 451-60

Oscarson M, Gullsten H, Rautio A, Bernal ML, Sinues B, Dahl ML, Stengard JH, Pelkonen O, Raunio H, Ingelman-Sundberg M (1998) Genotyping of human cytochrome P450 2A6 (CYP2A6), a nicotine C-oxidase. FEBS Lett 438: 201-5

Oscarson M, McLellan RA, Asp V, Ledesma M, Bernal Ruiz ML, Sinues B, Rautio A, Ingelman-Sundberg M (2002) Characterization of a novel CYP2A7/CYP2A6 hybrid allele (CYP2A6*12) that causes reduced CYP2A6 activity. Hum Mutat 20: 275-83

Oscarson M, McLellan RA, Gullsten H, Agundez JA, Benitez J, Rautio A, Raunio H, Pelkonen O, Ingelman-Sundberg M (1999a) Identification and characterisation of novel polymorphisms in the CYP2A locus: implications for nicotine metabolism. FEBS Lett 460: 321-7

Oscarson M, McLellan RA, Gullsten H, Yue QY, Lang MA, Bernal ML, Sinues B, Hirvonen A, Raunio H, Pelkonen O, Ingelman-Sundberg M (1999b) Characterisation and PCR-based detection of a CYP2A6 gene deletion found at a high frequency in a Chinese population. FEBS Lett 448: 105-10

Patterson F, Schnoll RA, Wileyto EP, Pinto A, Epstein LH, Shields PG, Hawk LW, Tyndale RF, Benowitz N, Lerman C (2008) Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther 84: 320-5

Pelkonen O, Rautio A, Raunio H, Pasanen M (2000) CYP2A6: a human coumarin 7-hydroxylase. Toxicology 144: 139-47

Perez-Stable EJ, Herrera B, Jacob P, 3rd, Benowitz NL (1998) Nicotine metabolism and intake in black and white smokers. JAMA 280: 152-6

Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ (1999) Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. J Pharmacol Exp Ther 289: 1545-52

Pfizer (2008) Pfizer Canada Inc. Product Monograph: Champix (varenicline tartrate tablets)

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391: 173-7

Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390: 401-4

Pitarque M, von Richter O, Oke B, Berkkan H, Oscarson M, Ingelman-Sundberg M (2001) Identification of a single nucleotide polymorphism in the TATA box of the CYP2A6 gene: impairment of its promoter activity. Biochem Biophys Res Commun 284: 455-60

Pitarque M, von Richter O, Rodriguez-Antona C, Wang J, Oscarson M, Ingelman-Sundberg M (2004) A nicotine C-oxidase gene (CYP2A6) polymorphism important for promoter activity. Hum Mutat 23: 258-66

Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382: 255-7

Quick MW, Lester RA (2002) Desensitization of neuronal nicotinic receptors. J Neurobiol 53: 457-78

Radloff L (1977) The CES-D scale: a self-report depression scale for research in the general population. Applied Psychological Measurement 1: 385-401

Rae JM, Johnson MD, Lippman ME, Flockhart DA (2001) Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. J Pharmacol Exp Ther 299: 849-57

Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM, Tyndale RF (2000) Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. Mol Pharmacol 58: 747-55

Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD, 3rd, Williams KE (2007) Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology 52: 985-94

Romberger DJ, Grant K (2004) Alcohol consumption and smoking status: the role of smoking cessation. Biomed Pharmacother 58: 77-83
Rossi S, Singer S, Shearman E, Sershen H, Lajtha A (2005) The effects of cholinergic and dopaminergic antagonists on nicotine-induced cerebral neurotransmitter changes. Neurochem Res 30: 541-58
Runkel M, Bourian M, Tegtmeier M, Legrum W (1997) The character of inhibition of the metabolism of 1,2-benzopyrone (coumarin) by grapefruit juice in human. Eur J Clin Pharmacol 53: 265-9
Scharf D, Shiffman S (2004) Are there gender differences in smoking cessation, with and without bupropion? Pooled- and meta-analyses of clinical trials of Bupropion SR. Addiction 99: 1462-9
Scherer G (1999) Smoking behaviour and compensation: a review of the literature. Psychopharmacology (Berl) 145: 1-20
Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C (2009) Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: A validation study. Pharmacol Biochem Behav 92: 6-11
Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF (2004) Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. Pharmacogenetics 14: 615-26
Schoedel KA, Sellers EM, Palmour R, Tyndale RF (2003) Down-regulation of hepatic nicotine metabolism and a CYP2A6-like enzyme in African green monkeys after long-term nicotine administration. Mol Pharmacol 63: 96-104
Schwartz RD, Kellar KJ (1985) In vivo regulation of [3H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J Neurochem 45: 427-33
Shields CB, Shields LB (2002) R. Glen spurling: surgeon, author, and neurosurgical visionary. J Neurosurg 96: 1147-53
Shields L (2002a) INR authors speak out: the effect of war on children. Int Nurs Rev 49: 71-2
Shields L (2002b) Muriel Knox Doherty--recognised at the 'Treasures' exhibition in Canberra. Collegian 9: 8
Shields L, Bryan B (2002) The effect of war on children: the children of Europe after World War II. Int Nurs Rev 49: 87-98
Shields L, Kristensson-Hallstrom I, Andershed B, Jackson K, Eriksson M (2002a) Nursing and health care in Sweden. Aust J Adv Nurs 20: 20-6
Shields LB, Hunsaker DM, Hunsaker JC, 3rd, Parker JC, Jr. (2002b) Sudden unexpected death in epilepsy: neuropathologic findings. Am J Forensic Med Pathol 23: 307-14
Shields LE, Lindton B, Andrews RG, Westgren M (2002c) Fetal hematopoietic stem cell transplantation: a challenge for the twenty-first century. J Hematother Stem Cell Res 11: 617-31
Shiffman S, Ferguson SG, Gwaltney CJ (2006a) Immediate hedonic response to smoking lapses: relationship to smoking relapse, and effects of nicotine replacement therapy. Psychopharmacology (Berl) 184: 608-18
Shiffman S, Scharf DM, Shadel WG, Gwaltney CJ, Dang Q, Paton SM, Clark DB (2006b) Analyzing milestones in smoking cessation: illustration in a nicotine patch trial in adult smokers. J Consult Clin Psychol 74: 276-85

Shimada T, Yamazaki H, Guengerich FP (1996) Ethnic-related differences in coumarin 7-hydroxylation activities catalyzed by cytochrome P4502A6 in liver microsomes of Japanese and Caucasian populations. Xenobiotica 26: 395-403
Smith SS, Jorenby DE, Leischow SJ, Nides MA, Rennard SI, Johnston JA, Jamerson B, Fiore MC, Baker TB (2003) Targeting smokers at increased risk for relapse: treating women and those with a history of depression. Nicotine Tob Res 5: 99-109
Spielberger C (1999) STAXI-2: the state trait anger expression inventory professional manual
Spitzer R, Williams JT, Gibbon M (1990) Structured Clinical Interview for DMS-III-R-Non-Patient Edition (SCID-NP, Version 1.0). American Psychiatric Press, American Psychiatric Press
SRNT (2002) Biochemical verification of tobacco use and cessation. Nicotine Tob Res 4: 149-59
Stapleton JA, Russell MA, Feyerabend C, Wiseman SM, Gustavsson G, Sawe U, Wiseman D (1995) Dose effects and predictors of outcome in a randomized trial of transdermal nicotine patches in general practice. Addiction 90: 31-42
Stavem K, Rogeberg OJ, Olsen JA, Boe J (2008) Properties of the Cigarette Dependence Scale and the Fagerstrom Test of Nicotine Dependence in a representative sample of smokers in Norway. Addiction 103: 1441-9
Stead LF, Perera R, Bullen C, Mant D, Lancaster T (2008) Nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev: CD000146
Stein EA, Pankiewicz J, Harsch HH, Cho JK, Fuller SA, Hoffmann RG, Hawkins M, Rao SM, Bandettini PA, Bloom AS (1998) Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. Am J Psychiatry 155: 1009-15
Strasser AA, Malaiyandi V, Hoffmann E, Tyndale RF, Lerman C (2007) An association of CYP2A6 genotype and smoking topography. Nicotine Tob Res 9: 511-8
Su T, Bao Z, Zhang QY, Smith TJ, Hong JY, Ding X (2000) Human cytochrome P450 CYP2A13: predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res 60: 5074-9
Surgeon's General Report (1988) The health consequences of smoking Surgeon General Report
National Center For Chronic Disease Prevention and Health Promotion. Atlanta, Georgia.
Swan GE, Carmelli D, Cardon LR (1996) The consumption of tobacco, alcohol, and coffee in Caucasian male twins: a multivariate genetic analysis. J Subst Abuse 8: 19-31
Swan GE, Carmelli D, Cardon LR (1997) Heavy consumption of cigarettes, alcohol and coffee in male twins. J Stud Alcohol 58: 182-90
Swan GE, Carmelli D, Rosenman RH, Fabsitz RR, Christian JC (1990) Smoking and alcohol consumption in adult male twins: genetic heritability and shared environmental influences. J Subst Abuse 2: 39-50
Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA (2004) Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 306: 1029-32
Tashkin D, Kanner R, Bailey W, Buist S, Anderson P, Nides M, Gonzales D, Dozier G, Patel MK, Jamerson B (2001) Smoking cessation in patients with chronic obstructive pulmonary disease: a double-blind, placebo-controlled, randomised trial. Lancet 357: 1571-5
Tiffany ST, Drobes DJ (1991) The development and initial validation of a questionnaire on smoking urges. Br J Addict 86: 1467-76

Tonnesen P, Paoletti P, Gustavsson G, Russell MA, Saracci R, Gulsvik A, Rijcken B, Sawe U (1999) Higher dosage nicotine patches increase one-year smoking cessation rates: results from the European CEASE trial. Collaborative European Anti-Smoking Evaluation. European Respiratory Society. Eur Respir J 13: 238-46

Tonstad S, Tonnesen P, Hajek P, Williams KE, Billing CB, Reeves KR (2006) Effect of maintenance therapy with varenicline on smoking cessation: a randomized controlled trial. Jama 296: 64-71

True WR, Heath AC, Scherrer JF, Waterman B, Goldberg J, Lin N, Eisen SA, Lyons MJ, Tsuang MT (1997) Genetic and environmental contributions to smoking. Addiction 92: 1277-87

Tsai ST, Cho HJ, Cheng HS, Kim CH, Hsueh KC, Billing CB, Jr., Williams KE (2007) A randomized, placebo-controlled trial of varenicline, a selective alpha4beta2 nicotinic acetylcholine receptor partial agonist, as a new therapy for smoking cessation in Asian smokers. Clin Ther 29: 1027-39

Vink JM, Beem AL, Posthuma D, Neale MC, Willemsen G, Kendler KS, Slagboom PE, Boomsma DI (2004) Linkage analysis of smoking initiation and quantity in Dutch sibling pairs. Pharmacogenomics J 4: 274-82

von Richter O, Pitarque M, Rodriguez-Antona C, Testa A, Mantovani R, Oscarson M, Ingelman-Sundberg M (2004) Polymorphic NF-Y dependent regulation of human nicotine C-oxidase (CYP2A6). Pharmacogenetics 14: 369-79

Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 54: 1063-70

West R, Hajek P, Foulds J, Nilsson F, May S, Meadows A (2000) A comparison of the abuse liability and dependence potential of nicotine patch, gum, spray and inhaler. Psychopharmacology (Berl) 149: 198-202

WHO (2008) WHO Report on the Global Tobacco Epidemic, 2008.

Williams KE, Reeves KR, Billing CB, Jr., Pennington AM, Gong J (2007) A double-blind study evaluating the long-term safety of varenicline for smoking cessation. Curr Med Res Opin 23: 793-801

Wise RA (2002) Brain reward circuitry: insights from unsensed incentives. Neuron 36: 229-40

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20: 92-8

Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, Eisen SA (2003) The heritability of failed smoking cessation and nicotine withdrawal in twins who smoked and attempted to quit. Nicotine Tob Res 5: 245-54

Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, Eisen SA (2005) Latent class typology of nicotine withdrawal: genetic contributions and association with failed smoking cessation and psychiatric disorders. Psychol Med 35: 409-19

Xu C, Rao YS, Xu B, Hoffmann E, Jones J, Sellers EM, Tyndale RF (2002) An in vivo pilot study characterizing the new CYP2A6*7, *8, and *10 alleles. Biochem Biophys Res Commun 290: 318-24

Yamanaka H, Nakajima M, Katoh M, Kanoh A, Tamura O, Ishibashi H, Yokoi T (2005) Trans-3'-hydroxycotinine O- and N-glucuronidations in human liver microsomes. Drug Metab Dispos 33: 23-30

Yamanaka H, Nakajima M, Nishimura K, Yoshida R, Fukami T, Katoh M, Yokoi T (2004) Metabolic profile of nicotine in subjects whose CYP2A6 gene is deleted. Eur J Pharm Sci 22: 419-25

Yamano S, Tatsuno J, Gonzalez FJ (1990) The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. Biochemistry 29: 1322-9
Yamazaki H, Inoue K, Hashimoto M, Shimada T (1999) Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. Arch Toxicol 73: 65-70
Yamazaki H, Inui Y, Yun CH, Guengerich FP, Shimada T (1992) Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. Carcinogenesis 13: 1789-94
Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT, Yokoi T (2003) Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity in vivo and in vitro. Clin Pharmacol Ther 74: 69-76
Yoshida R, Nakajima M, Watanabe Y, Kwon JT, Yokoi T (2002) Genetic polymorphisms in human CYP2A6 gene causing impaired nicotine metabolism. Br J Clin Pharmacol 54: 511-7
Zacny JP, Stitzer ML (1988) Cigarette brand-switching: effects on smoke exposure and smoking behavior. J Pharmacol Exp Ther 246: 619-27
Zevin S, Jacob P, 3rd, Benowitz N (1997) Cotinine effects on nicotine metabolism. Clin Pharmacol Ther 61: 649-54
Zhang X, D'Agostino J, Wu H, Zhang QY, von Weymarn L, Murphy SE, Ding X (2007) CYP2A13: variable expression and role in human lung microsomal metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. J Pharmacol Exp Ther 323: 570-8