Pharmacogenetics of smoking cessation: role of nicotine target and metabolism genes

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Abstract

Many smokers attempt to quit smoking but few are successful in the long term. The heritability of nicotine addiction and smoking relapse have been documented, and research is focused on identifying specific genetic influences on the ability to quit smoking and response to specific medications. Research in genetically modified cell lines and mice has identified nicotine acetylcholine receptor subtypes that mediate the pharmacological and behavioral effects of nicotine sensitivity and withdrawal. Human genetic association studies have identified single nucleotide polymorphisms (SNPs) in genes encoding nicotine acetylcholine receptor subunits and nicotine metabolizing enzymes that influence smoking cessation phenotypes. There is initial promising evidence for a role in smoking cessation for SNPs in the β2 and α5/α3/β4 nAChR subunit genes; however, effects are small and not consistently replicated. There are reproducible and clinically significant associations of genotypic and phenotypic measures of CYP2A6 enzyme activity and nicotine metabolic rate with smoking cessation as well as response to nicotine replacement therapies and bupropion. Prospective clinical trials to identify associations of genetic variants and gene–gene interactions on smoking cessation are needed to generate the evidence base for both medication development and targeted therapy approaches based on genotype.

Introduction

Cigarette smoking continues to be the leading cause of preventable deaths in the United States, accounting for 1 in 5 deaths every year (CDC 2010). In 2009, 20.6% of US adults were current cigarette smokers (CDC 2010). Many smokers are aware of the harmful effects of smoking and about 70% of smokers report wanting to quit completely (CDC 2010); however, only 4–7% of smokers are able to quit smoking without medication or formal treatment (Fiore et al. 2008). Yet, recent data also suggest that only about 22% of smokers who attempted to quit used cessation medication (Cokkinides et al. 2005; Fiore et al. 2008). Among those smokers who do use pharmacotherapy, fewer than one-third are able to stay smoke-free for at least 6 months (Fiore et al. 2008).

FDA-approved approaches for smoking cessation include nicotine replacement therapy (NRT), bupropion, and varenicline. NRTs include: transdermal patch, nicotine gum, inhalers, lozenges, and nasal spray. Meta-analyses of clinical trial efficacy data indicate that

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NRTs double the odds of successful quitting for at least 6 months compared to placebo (Moore et al. 2009; Stead et al. 2008). Bupropion, an anti-depressant medication, is a norepinephrine-dopamine reuptake inhibitor (Learned-Coughlin et al. 2003; Terry and Katz 1997) as well as a nicotinic acetylcholine receptor antagonist (Slemmer et al. 2000). Bupropion has similar efficacy to NRT and data from a meta-analysis revealed that bupropion doubles abstinence rates for at least 6 months compared to placebo (Hughes et al. 2007). Varenicline, an α4β2* nicotinic acetylcholine receptor partial agonist, was developed to mimic the effects of nicotine by stimulating a moderate amount of dopamine release to prevent craving and withdrawal, while reducing the reinforcing effects of nicotine from cigarette smoking (Rollema et al. 2007). Meta-analysis data indicate that varenicline improves the odds of abstinence two to three-fold, relative to placebo (Cahill et al. 2011). Varenicline is more effective for smoking cessation than NRTs (Aubin et al. 2008; Wu et al. 2006b) or bupropion (Gonzales et al. 2006). Yet, even with the most efficacious pharmacotherapies, the majority of smokers are unable to quit and maintain abstinence (Schnoll and Lerman 2006).

Quitting smoking is very difficult due to nicotine's addictive properties. There is substantial variability in nicotine addiction and relapse rates which is attributable, in part, to heritable influences. Twin studies estimate that heritable influences account for roughly 46–84% of the variability in smoking initiation and smoking persistence and as much as 75% of the variability in nicotine dependence (Sullivan and Kendler 1999; Vink et al. 2005). Other studies have shown that heritable factors account for 29–53% of the variability in withdrawal symptoms and 51–54% of the variance in quitting success (Lessov et al. 2004; Pergadia et al. 2006; Xian et al. 2003, 2005).

Research has focused on identifying specific genetic influences on the ability to quit smoking. Such research can identify novel molecular targets for medication development. Further, pharmacogenomics research focused on genetic influences on quitting success with specific medications could potentially lead to the development of targeted therapy approaches that are more efficacious. This review focuses on the role in smoking cessation of genes coding for neuronal nicotinic acetylcholine receptors (nAChRs) and nicotine metabolizing enzymes, the initial molecular targets for nicotine's effects. First, we provide a brief overview of the neurobiology of nicotine's effects in the brain, phenotyping of smoking cessation behaviors, and approaches for genetic studies. Next, we review the biological plausibility and available evidence for the association of specific cholinergic genes with smoking cessation and therapeutic response. Lastly, we review nicotine pharmacokinetics and the role of genetic variability in nicotine metabolism enzymes.

Neurobiology of nicotine

Nicotinic acetylcholine receptors

Neuronal nAChRs are heterogeneous cationic channels that are widely distributed in the nervous system and non-neuronal tissues. The subunit composition and biophysical properties of nAChRs depend on the subtype and the area of the brain in which they are found (Gaimarri et al. 2007; Gotti et al. 2007; Zoli et al. 2002). Nicotine diffuses readily into the brain and binds to ligand-gated nAChRs. After nicotine binds to the nAChR, the channel opens and allows entry of cations (Na^+, Ca^{2+}).

nAChRs are homopentameric or heteropentameric units that are localized primarily at presynaptic or preterminal sites, where they modulate neurotransmitter release. They are also found on cell bodies and dendrites, where they mediate postsynaptic effects. nAChRs have an important role in regulating transmitter release, cell excitability, and neuronal integration (Gotti and Clementi 2004; Hogg and Bertrand 2004; Hogg et al. 2003).

nAChRs form a family of subtypes formed by five subunits (Gotti and Clementi 2004; Gotti et al. 2006). The subunits are encoded by nine (α2–α10) and three (β2–β4) subunit genes and the two major subfamilies are classified as either αBgtx-sensitive or αBgtx-insensitive. αBgtx-sensitive nAChRs are mostly homopentameric and the αBgtx-insensitive nAChRs are only heteropentameric, consisting of both α and β subunits. αBgtx-insensitive nAChRs are mostly comprised of the α4β2* subtype and may include a primary α6 or accessory α5 or β3 subunits. α5 and β3 do not form functional channels alone, but combined with other α-β combinations, they contribute to receptor targeting and localization in neuronal plasma membrane domains, and modify other receptor properties (Gotti et al. 2006, 2007).

Neurotransmitter pathways and nicotine dependence

There is a well-supported role of nicotine as the pharmacological agent that maintains dependence. The composition, anatomical localization, and physiological properties of nAChRs contribute to various physiological and behavioral differences (Govind et al. 2009; Marks et al. 1999). Nicotine and other nicotine agonists will elicit different responses of these receptors depending on the subtype it acts on (Brioni et al. 1997; Changeux 2010; Govind et al. 2009). Nicotine stimulates release of dopamine in the mesoaccumbens reward pathway (Champtiaux et al. 2003) and dependence is mediated, in part, by activation of specific subtypes of nAChRs on dopamine neurons (Court et al. 1998; Picciotto and Corrigall 2002). Dopamine release signals a pleasurable experience and is a key component of the reinforcing effects of nicotine and other drugs of abuse (Changeux 2010; Nestler 2005). Via nAChRs, nicotine activates both dopamine and GABA neurons of the ventral tegmental area (VTA), resulting in dopamine release in the nucleus accumbens and contributing to the rewarding and motivational effects of nicotine (Corrigall et al. 1994; Laviolette and van der Kooy 2004; Mineur and Picciotto 2008; Nisell et al. 1994). Further, molecular alterations in central dopamine systems contribute to craving and modulate the aversive effects of nicotine withdrawal (Changeux 2010; Laviolette and van der Kooy 2004; Mansvelder et al. 2002; Mansvelder and McGehee 2002). While a single cigarette smoked in the morning can saturate and desensitize roughly 80–90% of nAChRs for several hours, smokers may continue to smoke throughout the day to avoid having free unbound receptors (Brody et al. 2006). The free, non-desensitized receptors may be responsible for cravings and continued smoking acts on these receptors to provide positive reinforcement and sustained dopamine release to prevent withdrawal symptoms (Balfour 2004; Brody et al. 2006).

Smoking cessation phenotypes

A well-defined phenotype is crucial to the success of genetics research. Smoking cessation, the focus of this review, is simply defined as the ability to maintain long-term abstinence (Silagy et al. 2004). Effective cessation occurs by overcoming different milestones to achieve persistent abstinence, including a period of initial abstinence (24 h without smoking), avoiding lapse (first use after abstinence), and ultimately avoiding relapse (proceeding from first use after abstinence back to regular daily use) (Shiffman et al. 2006). The inability to maintain abstinence is related to the severity of withdrawal symptoms as well as genetic factors and influenced, in part, by psychological factors and contextual cues (Dawkins et al. 2009; Pomerleau et al. 2005; Xu et al. 2008).

Nicotine dependence is associated with decreased rates of initial cessation and a higher risk of transitioning from lapse to relapse (Japuntich et al. 2011). Although pre-quit level of nicotine dependence is related to relapse, it is not a consistent predictor of quit attempts and smoking cessation, and that association is highly dependent on which measurement is used (Etter 2005; Japuntich et al. 2011; Piper et al. 2006). For example, the widely used Fagerstrom Test for Nicotine Dependence (FTND) (Fagerstrom 1978; Heatherton et al.

1991) does not consistently and robustly predict withdrawal symptoms or quitting success (Borrelli et al. 2001; Piper et al. 2004; Silagy et al. 1994). The same is true for measures of smoking heaviness predicting cessation (Fidler et al. 2011). Together with evidence suggesting that nicotine dependence and smoking cessation each have some unique genetic contributions (Li et al. 2003; Xian et al. 2003), these data underscore the point that nicotine dependence and smoking cessation represent distinct phenotypes. While some genetic influences may be shared by these phenotypes, it cannot be assumed that genetic associations with nicotine dependence will translate to smoking cessation or vice versa.

Given the limited variability in successful quitting predicted by smoking behavior and nicotine dependence, genetics studies are focusing increasingly on the smoking cessation phenotype. Retrospective cross-sectional measures of cessation, comparing former smokers to current smokers, have been used with some success (Breitling et al. 2009a). A more refined phenotype for smoking cessation is based on ability to quit among treatment-seeking smokers followed prospectively in longitudinal observational studies or clinical trials (Hughes et al. 2010). While retrospective measures tend to be less precise than longitudinal measures, they can be collected more efficiently on larger populations.

Genetic research approaches

Nicotine dependence involves complex interactions between multiple genes and environmental factors (Li 2008). There are a wide range of research approaches to understand the genetic influences on nicotine dependence, ranging from transgenic animal models to population studies. Pharmacological and molecular biology studies include in vitro receptor genetic mutations, as well as knock-out (KO) and point mutation knock-in animal models (Greenbaum and Lerer 2009). These studies evaluate the pharmacological and biochemical properties of specific gene manipulations to determine their contribution to nicotine dependence and/or nicotine withdrawal.

Efforts to discover chromosomal regions, and ultimately genes associated with a specific phenotype or disease like nicotine dependence, can utilize linkage analysis. Genes located near each other on the same chromosome will likely be inherited together. Linkage analysis assesses the presence of a phenotype in large, high-risk families, with the goal of determining the location of the gene responsible for the phenotype in relation to a known genetic marker. Linkage analysis studies can be very useful in identifying the location of a susceptibility disease gene, but there are several limitations. Largely, the results of linkage analyses of nicotine dependence have not been consistently replicated by more than two independent studies and there is a great deal of variability in the measures that are used to assess nicotine dependence (Han et al. 2010; Li 2008). However, of the 13 regions on 11 chromosomes that have been identified in nicotine dependence linkage analyses, regions on chromosomes 9, 10, 11, and 17 have been detected by the greatest number of studies; chromosomes 1, 5, 10, 11, 12, 16, 20, and 22 have also shown some linkage signals (Li 2008). Overall, linkage analysis has identified several different loci that are linked to nicotine addiction and has been a starting point for identification of genes that may influence nicotine dependence phenotypes (Han et al. 2010). The results of linkage analyses have, in some cases, led to candidate gene association studies of nicotine dependence phenotypes.

Candidate gene association studies investigate single nucleotide polymorphisms (SNPs) and other variants (e.g., variable number tandem repeats) in genes suggested in linkage analysis (Hernandez and Blazer 2006), and/or those with strong biological plausibility. These studies can compare unrelated groups (case–control) or members of family units (family-based) and may be useful in identifying genes that play a minor role in nicotine dependence (Portugal and Gould 2008; Risch and Merikangas 1996). Until recently, most studies have focused on

a few select candidate gene regions and it is possible that many of the loci that influence smoking behavior may lie outside those previously studied regions (Caporaso et al. 2009). Other limitations include false positive results (Sullivan et al. 2001), small sample sizes, differences in measures of smoking behavior, and differences in ethnic and environmental backgrounds (Caporaso et al. 2009; Portugal and Gould 2008).

Pathway-based association studies are a useful tool to study the associations of multiple genes within a neurobiological pathway or system that has strong biological plausibility for association with a phenotype. If amply powered, such studies can account for multiple variants in interacting or related genes and may help to validate the role of SNPs or genes in the etiology of a nicotine dependence phenotype (Conti et al. 2008; Saccone et al. 2007; Wang et al. 2007). Wang and Li identified several pathways (calcium, dopamine receptor, and cAMP-mediated signaling) containing genes associated with smoking behavior phenotypes, including initiation, dependence, and cessation. There is genetic overlap in the main pathways identified for smoking behavior phenotypes, but the genes associated with each phenotype within a pathway are different (Wang and Li 2010). Many pathways important in addiction to cocaine, alcohol, opioids overlap with those important in nicotine addiction or smoking cessation, including gap junction, LTP, and MAPK signaling (Li 2008). These findings suggest that some but not all of the mechanisms underlying nicotine dependence are common with other substances in addiction. There are likely many genetic targets in other neuronal pathways that affect smoking behavior (Munafo and Johnstone 2008); therefore a pathway-based approach may be restricted by the large number of genes that contribute to other disease phenotypes and those for which a biological function is still unknown (Elbers et al. 2009; Munafo and Johnstone 2008; Wei et al. 2010). A pathway-based approach may help in the interpretation of genome-wide association data and lead to identification of novel mechanisms and genes contributing to smoking behavior (Wang et al. 2007; Wang and Li 2010).

In contrast to candidate gene and pathway-based approaches, genome-wide association studies (GWAS) are hypothesis-free and seek to identify loci for novel susceptibility genes. Such approaches have discovered genetic variants in the α5/α3/β4 nAChR gene cluster as associated with nicotine dependence and heaviness of smoking (Berrettini et al. 2008; Bierut et al. 2008; Thorgeirsson and Stefansson 2008). These genes have attracted a significant amount of attention and the associations with smoking heaviness are robust and important. However, studies attempting to confirm associations of these SNPs with retrospective and prospective smoking cessation have yielded mixed results (Breitling et al. 2009a; Greenbaum and Lerer 2009; Ray et al. 2010). While GWAS is a very useful approach, genetic variants identified via GWAS account for a very small proportion of the variance in smoking behavior (<5%), and thus, the clinical utility of these results is not yet clear (Furberg et al. 2010). To date, few GWAS studies of smoking cessation have been conducted (Uhl et al. 2007) in part due to the need for larger sample sizes than may be feasible to acquire from clinical trials. Below, we review genetic studies of the smoking cessation phenotypes with a focus on the role of genes encoding nAChR subunits and those implicated in nicotine metabolism.

Nicotinic acetylcholine receptor (nAChR) subunits

CHRNA5- CHRNA3- CHRNB4

The 15q24 region on chromosome 15 includes the genes *(CHRNA5-CHRNA3-CHRNB4)* that encode the α5, α3, and β4 nAChR subunits, which are in strong linkage disequilibrium (LD) with each other. Several recent GWAS and pathway-based studies have identified SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster as associated with heaviness of

smoking and/or nicotine dependence (Berrettini et al. 2008; Bierut et al. 2007; Caporaso et al. 2009; Saccone et al. 2007; TAG 2010; Thorgeirsson and Stefansson 2008).

The α5 subunit does not form functional receptors when expressed alone, or co-expressed with a β subunit (Ramirez-Latorre et al. 1996), but when combined with an additional α subunit and either a β2 or β4 subunit it alters several pharmacological properties of the receptor in response to nicotine (Gerzanich et al. 1998; Tapia et al. 2007). The α5 variant D398N, in the region encoding the α5, α3, and β4 nAChR subunits, causes a change in the amino acid sequence and has been shown to reduce the response to epibatidine of $(\alpha4\beta2)_2\alpha5$ nAChRs, a subtype important in mediating the effects of nicotine (Bierut et al. 2008). Kuryatov and others expressed this variant in *X. laevis oocytes* and observed that the α5 Asn 398 risk variant in $(\alpha4\beta2)_2\alpha5$ nAChRs had lower Ca^{2+} permeability and greater short-term desensitization than the α5 Asp 398 variant when exposed to nicotine. The results suggest that this variation in the α5 subtype might reduce nicotinic signaling, potentially resulting in heavier smoking (Kuryatov et al. 2011).

These in vitro studies are being extended in transgenic mouse model studies to clarify the behavioral mechanisms underlying these associations. For example, α5 knock-out mice respond for higher doses of intravenously self-administered nicotine infusions compared to wild-type mice, suggesting that the α5 subunit may be important in the experience of toxicity associated with high nicotine doses (Fowler et al. 2011). In another study, α5 KO mice were implanted with osmotic mini-pumps containing nicotine for 14 days, followed by precipitated withdrawal with mecamylamine (Jackson et al. 2008). The α5 KO mice showed a reduction in somatic signs only, suggesting that this subtype is involved with the physical measures of withdrawal symptoms but not affective withdrawal signs (Jackson et al. 2008). Salas and colleagues examined α5 KO mice chronically treated with nicotine by osmotic mini-pumps or in their drinking water, followed by mecamylamine precipitated withdrawal. Consistent with the results of Jackson and colleagues, the lack of the α5 subunit abolished the somatic symptoms of withdrawal to levels exhibited by saline-treated mice, suggesting that the α5 subunit mediates some physical symptoms of nicotine withdrawal (Jackson et al. 2008; Salas et al. 2009). However, because physical symptoms are a less significant component of the withdrawal syndrome in human smokers compared to affective and cognitive signs, the relevance in humans must be evaluated.

The α3 and β4 subunits are most commonly expressed together in the same receptor subtype, with or without α5, in the peripheral nervous system (Wang et al. 1996). The β4 subunit is also found co-expressed with other α subunits in the central nervous system, with high expression in the medial habenula and interpeduncular nucleus (Salas et al. 2003). α3 and β4 containing receptors are involved in nicotine-induced seizures (Salas et al. 2004a) and β4 receptors may be important in anxiety-related behaviors (Salas et al. 2003). Oocytes containing β4 subunit polymorphisms showed varying differences in the EC_{50} for ACh activation (Liang et al. 2005). One variant, present in only the Caucasian sample, had a lower sensitivity to ACh, increased ACh EC_{50}, and the slowest onset of desensitization. The other variants, found only in the African-American sample, had a higher sensitivity to ACh and lower EC_{50} (Liang et al. 2005). Mice null for the β4 nAChR subunit implanted with 13-day osmotic mini-pumps displayed significantly reduced mecamylamine-induced somatic (shaking, grooming, scratching, chewing) and non-somatic (hyperalgesia) symptoms of withdrawal, suggesting a role for this subunit in the appearance of nicotine withdrawal symptoms (Salas et al. 2004b).

Evidence of the involvement of the α5, α3, and β4 subunits in nACh receptor desensitization rates, calcium permeability, and response to nicotine (Gerzanich et al. 1998; Tapia et al. 2007), as well as the association of the *CHRNA5-CHRNA3-CHRNB4* gene cluster with

nicotine dependence phenotypes, has led to studies of the role of key SNPs in withdrawal symptoms and smoking cessation. In a case–control study of 1,446 current versus former smokers, no evidence was found for associations of SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster with retrospective smoking cessation (Breitling et al. 2009a). Similarly two independent nAChR systems-based analyses found no evidence for associations of SNPs in this gene cluster with prospective smoking cessation in participants treated with transdermal NRT or bupropion (Conti et al. 2008; Ray et al. 2010). However, data published by Baker and others provides evidence for association of *CHRNA5-CHRNA3-CHRNB4* haplotypes with tolerance, craving, and loss of control, but only among individuals who began smoking early in life, suggesting that the genetic risk may only occur with early tobacco exposure (Baker et al. 2009). The *CHRNA5-CHRNA3-CHRNB4* haplotypes are also associated with withdrawal severity and inability to stop smoking, but there was no interaction with age at daily smoking for these phenotypes (Baker et al. 2009). Another study by Sarginson and colleagues identified *CHRNA5* SNP rs680244 and *CHRNB4 SNP rs*12914008 as predictors of 7-day point-prevalence at 52-week follow-up in Caucasians and the *CHRNA3* SNP rs8192475 as a predictor of craving after quitting and withdrawal symptoms over 42 days (Sarginson et al. 2011). More recently, Munafo and colleagues found a weak association between the rs1051730 polymorphism and decreased likelihood of smoking cessation at follow-up after 4 weeks of open-label nicotine transdermal patch treatment, but not in a second placebo-controlled clinical trial (Munafo et al. 2011). Recent data from King and colleagues show an association of *CHRNA5* SNP rs518425 with abstinence in smokers treated with the $\alpha 4\beta 2^*$ partial agonist varenicline in a large pharmacogenetic trial (King et al. 2011).

Most smoking behavior genetic studies limit their sample populations to European ancestry, but association studies in diverse populations with different linkage disequilibrium patterns and alternate phenotypes may enhance the search for variants relevant to smoking behavior. Li and colleagues reported data from the first study of the association of SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster with smoking behavior in a large Korean sample (Li et al. 2010). Smoking cessation, defined based on smoking status as former versus current smoker, was associated with the *CHRNB4* SNP rs11072768 as well as with haplotypes in this gene cluster (Li et al. 2010). Hamidovic et al. (2011) conducted a study in 1,710 African-American current or former smokers. They evaluated smoking persistence, which accounts for periods of smoking cessation during a larger smoking timeframe. *CHRNA5* SNP rs12915366 and *CHRNA3* SNP rs12914385 were found to be associated with smoking persistence (Hamidovic et al. 2011). Both SNPs are located in regions that have been associated with smoking and nicotine dependence in European Americans (Liu et al. 2010; Saccone et al. 2009; TAG 2010), suggesting that there may be distinct variants which modulate smoking behavior phenotypes in African-Americans. Smoking during pregnancy is a phenotype influenced by many factors, including genetic susceptibility to the addictive properties of nicotine. Data from 2,474 European women, who smoked regularly before becoming pregnant, showed that the risk allele of the rs1051730 SNP in the *CHRNA5-CHRNA3-CHRNB4* gene cluster is associated with continuing to smoke during pregnancy (Freathy et al. 2009). Thorgeirsson and Stefansson (2010) replicated these findings, finding the rs1051730 risk variant is also associated with continued smoking during pregnancy in their sample of 1,891 women.

The reviewed research on the *CHRNA5-CHRNA3-CHRNB4* gene cluster in smoking cessation phenotypes from animal and human genetic data suggests that variability in these subunit genes may have some small contribution to the physical withdrawal symptoms and the ability to abstain from smoking. The role of variation in the *CHRNA5-CHRNA3-CHRNB4* gene cluster may be important in determining response to treatments for smoking cessation; however, this hypothesis has not been tested extensively. Ethnicity and smoking

behavior phenotypes may be critical factors in determining the significance of associations between this gene cluster and smoking cessation and cessation treatment response.

CHRNA6- CHRNB3

α6-containing nAChRs are expressed mainly in brain areas that are involved in nicotine-stimulated dopamine release (Champtiaux et al. 2003; Lai et al. 2005; Salminen et al. 2004), and have been implicated in mediating nicotine dependence (Laviolette and van der Kooy 2004). One of the first studies examining the pharmacology of α6-containing nAChRs generated mice lacking the α6 subunit gene (Champtiaux et al. 2002). Null mutant mice had no high-affinity α-conotoxin MII-sensitive (α6 selective antagonist) binding sites (Champtiaux et al. 2002; Drenan et al. 2008; Yang et al. 2009). A majority of β3 subunits are assembled with α6 subunits (Champtiaux et al. 2003; Yang et al. 2009) and chronic nicotine treatment decreases α6-containing nAChRs in the striatum but does not change the density of α6 nAChRs that contain β3 subunits (Perry et al. 2007). Jackson and others measured physical (somatic and hyperalgesia) and affective [anxiety-related behavior and conditioned place aversion (CPA)] nicotine withdrawal behaviors in C57BL/6J mice implanted with osmotic mini-pumps filled with nicotine or saline for 14 days (28 days for CPA). Withdrawal was initiated after 14 days of nicotine infusion or by removal of the mini-pumps. On day 15, mice were injected intracerebrovascularly with α6-selective antagonist α-conotoxin MII(H9A;L15A) and withdrawal signs were measured 10 min after injection. α-conotoxin MII(H9A;L15A) blocked withdrawal-associated CPA and anxiety-related behaviors (elevated plus maze) compared to mice injected intracerebroventricularly with saline, suggesting a role for the α6 subunit in affective nicotine withdrawal symptoms (Jackson et al. 2009). This study also demonstrated a role for the α6 subunit in nicotine reward, reflecting possible differences in nAChR subunit composition (α4α6β2* vs. α6β2* vs. α4α6α5β2*), different anatomical distribution of brain nAChRs, and/or different intracellular mechanism in nicotine reward and withdrawal (Jackson et al. 2009). α6* nAChRs are located in both the VTA and locus coeruleus. The VTA has been implicated in both nicotine reinforcement (Corrigall et al. 1992; Pons et al. 2008) and withdrawal (Bruijnzeel and Markou 2004) and the locus coeruleus has been implicated in withdrawal effects from drugs of abuse (Dizgah et al. 2005), implying that the contributions of α6* nAChRs to nicotine reward are mediated by the VTA, but α6* nAChRs in the locus coeruleus may be responsible for regulating nicotine withdrawal.

Genetic association studies have shown that SNPs in the genes encoding the α6 and β3 subunits, *CHRNA6* and *CHRNB3*, are associated with nicotine dependence and subjective response to nicotine (Bierut et al. 2007; Greenbaum et al. 2006; Saccone et al. 2007; Zeiger et al. 2008). The role of *CHRNA6* and *CHRNB3* in smoking cessation was examined by Hoft et al. (2009). They used a family-based approach, genotyping 1,051 subjects (50% members of sibling pairs), to test for an association with quit attempts and overall dependence as measured by DSM-IV dependence scale. The rs7004381, rs4950 and, rs13280604 SNPs in the *CHRNB3* gene were associated with self-reported number of unsuccessful quit attempts, while *CHRNA6* SNP rs2304297 was associated with nicotine dependence. Replication of associations between specific SNPs and smoking behavior phenotypes has not been easy due to inconsistencies in the definition of a phenotype and the inclusion sample. Hoft et al. (2009) performed secondary analyses with sets of individuals who reported having used tobacco every day for at least 1 month in their lives and the entire sample of 1,051 individuals. The found that analysis using different subsets of the sample did in fact show that sample selection affected the ability to detect an association (Hoft et al. 2009).

It is necessary for future studies to understand the molecular and functional differences of various SNPs in both the *CHRNA6* and *CHRNB3* genes to be able to elucidate their roles in nicotine dependence and determine if they contribute to the ability to abstain from smoking.

CHRNA4

CHRNA4 is the gene that encodes the α4 nAChR subunit. The majority of α4 subunits form functional nAChR complexes with at least one β2 subunit and together they account for more than 90% of the high-affinity nicotinic receptors in the brain (Whiting and Lindstrom 1986). α4β2* receptors are expressed in many CNS regions, and α4 mRNA is expressed in nearly every dopaminergic and GABAergic neuron in the VTA, the region affecting dopamine release into the nucleus accumbens. The α4 subunit plays a role in the reinforcing effects of nicotine (Klink et al. 2001), tolerance, and the modulation of mesolimbic dopamine function, all essential to the development of the nicotine dependence phenotype (Tapper et al. 2004).

Mice lacking the α4 subunit generated by Marubio et al. (1999) no longer express high-affinity binding sites. α4 KO mice also show a reduced antinociceptive effect of nicotine on the hot-plate test and diminished sensitivity to nicotine in the tail-flick test. Tapper and colleagues generated knock-in mice that express the Leu9′Ser mutation, expressing a receptor hypersensitive to nicotine. They found that selective activation of these receptors with low doses of nicotine replicates tolerance and sensitization stimulated by chronic nicotine administration, suggesting a role for this subunit in various smoking behavior phenotypes (Tapper et al. 2004). This mutant mouse model was also used by Labarca et al. (2001) and others who found that the hypersensitive mice display increased anxiety and poor motor learning, which is eliminated by low levels of nicotine administered i.p. Quantification of nAChR subunit gene expression indicates that posterior ventral tegmental area (pVTA) neurons express higher levels of α4 (as well as α6 and β3) transcripts than anterior VTA neurons (Zhao-Shea et al. 2011). Infusion of α-conotoxin MII into the VTA blocked activation of pVTA dopaminergic neurons in wild-type and α4* Leu9'Ala knock-in mice, indicating that nicotine selectively activates dopaminergic neurons within the pVTA through α4α6* nAChRs (Zhao-Shea et al. 2011).

The *A529T* polymorphism in the *CHRNA4* gene has been shown to influence varying responses to nicotine and it was found that mice strains with the *A529T* genotype consumed less nicotine (in solution) and preferred solutions with a lower concentration of nicotine (Butt et al. 2005; Stitzel et al. 2000; Tritto et al. 2002). Wilking and others found that *CHRNA4* A529T knock-in mice exhibited greater sensitivity to the hypothermic effects of nicotine, consumed less nicotine, and did not develop conditioned place preference. *A529T* mice produced a smaller maximal functional response to acetylcholine (ACh) than *T529A* littermates and the percent of response to ACh by high sensitivity α4* nAChRs was significantly greater for nAChRs containing the *A529T* variant (Wilking et al. 2010).

In human studies, SNPs or haplotypes of the *CHRNA4* gene have been associated with nicotine dependence in family-based analyses (Feng et al. 2004; Li et al. 2005), a large-scale genetic association study (Breitling et al. 2009b), and in a study evaluating unrelated European Americans and African-Americans (Han et al. 2011). GWAS studies have not identified the *CHRNA4* gene as a risk locus for smoking behavior phenotypes (Berrettini et al. 2008; Caporaso et al. 2009; Liu et al. 2009, 2010; TAG 2010; Thorgeirsson et al. 2010; Thorgeirsson and Stefansson 2008; Vink et al. 2009). However, in a candidate gene study, the rs2236196 polymorphism was associated with smoking cessation outcomes (Hutchison et al. 2007). Specifically, this SNP was associated with self-reported abstinence for 7 days before the end of 8 weeks of treatment with either transdermal nicotine patch or nicotine nasal spray. An independent sample of smokers with the rs2236196 polymorphism reported

increased sensitivity to the acute effects of smoking after 8 h of abstinence and subjects treated with nasal spray maintained treatment gains at 6 months follow-up (Hutchison et al. 2007). In addition, *CHRNA4* SNP rs2236196, as well as rs3787138 and rs6062899, were associated with abstinence in smokers treated with varenicline, consistent with varenicline's molecular target (King et al. 2011). rs2236196 is located in the 3′ end of the untranslated region of the *CHRNA4* gene and alters an mRNA biding site for the iron-responsive element (IRE). Nucleotide substitutions lead to a significant loss of binding affinity for IRE (Erlitzki et al. 2002; Hentze et al. 2004; Jaffrey et al. 1993) and IREs have been found to regulate translation of genes involved in CNS function (Rogers et al. 2002). The association of *rs2236196* with changes in sensitivity to the acute effects of nicotine and with response to nicotine therapy may be a result of changes at the protein level, which may lead to a change in CNS sensitivity to cholinergic stimulation (Hutchison et al. 2007).

Evidence from animal and human studies supports a role for variation in the *CHRNA4* gene in altering the sensitivity of α4-containing nAChRs and response to NRT. Initial evidence suggests that *CHRNA4* variation may alter sensitivity to nicotine and quitting success with forms of NRT that reach the CNS more quickly (nasal spray). Yet, these data have yet to be replicated and more work is needed to identify functional SNPs that may yield more robust and reproducible associations with cessation phenotypes.

CHRNB2

nAChRs that contain both α4 and β2 subunits are the most abundant in the brain, and nicotine has the highest affinity for α4β2* receptors (Benowitz et al. 1989; Flores et al. 1992; Wu et al. 2006a). Recent evidence suggests that nAChRs containing the β2 subunit determines the sensitivity to nicotine (Marubio et al. 1999; McCallum et al. 2006; Tritto et al. 2004) and are critical for many of the reinforcing properties of nicotine (Nashmi and Lester 2006; Picciotto et al. 1998; Whiting and Lindstrom 1986), but may not play a role in nicotine withdrawal symptoms in rodents (Besson et al. 2006). The VTA has been shown to mediate most of the positive motivational effects of nicotine in rats (Laviolette and van der Kooy 2004) and most dopaminergic neurons in the VTA express β2* containing nAChRs (Klink et al. 2001) and upregulation of nAChRs by nicotine is restricted to nAChRs containing the β2 subunit (Davila-Garcia et al. 2003; Flores et al. 1997, 1992; Mao et al. 2008; Marks et al. 2004; McCallum et al. 2006; Nguyen et al. 2003).

Knockout mice that lack the β2 subunit do not self-administer nicotine and do not show nicotine-stimulated dopamine release in the ventral striatum, implicating its role in mediating nicotine dependence (Besson et al. 2006; Picciotto et al. 1998), however, the β2 KO mice exhibit normal withdrawal symptoms (increased levels of rearing and jumping) (Besson et al. 2006). Marubio et al. (1999) showed that β2 KO mice display a reduction in high-affinity binding sites, a reduced antinociceptive effect to nicotine on the hot-plate test, and diminished sensitivity to nicotine in the tail-flick test, supporting a role for this subunit in the antinociceptive effects of nicotine.

The affective and cognitive signs of nicotine withdrawal are believed to be an important factor in failed cessation attempts and continued use (Koob et al. 1993; Markou et al. 1998). Jackson et al. (2008) measured affective signs of withdrawal and found a loss of anxiety-related behavior and loss of aversion in v2 KO mice. Cognitive withdrawal symptoms, as assessed by the contextual fear conditioning paradigm, were examined in β2 KO mice by Portugal et al. (2008). v2 KO mice withdrawn from nicotine showed no withdrawal-related deficits on contextual fear conditioning (Portugal and Gould 2008) compared to deficits exhibited in wild-type mice. Further, nicotine acting on intact hippocampal β2-containing nAChRs may alter hippocampal function accounting for the withdrawal deficits in contextual fear conditioning (Davis and Gould 2007). Subsequently, Raybuck and Gould

found that nicotine withdrawal in β2 KO mice did not produce the trace fear conditioning deficits observed in wild-type mice. Evidence from these studies suggests an important role for β2 containing nAChRs in withdrawal-related deficits in learning and memory (Raybuck and Gould 2009).

There is evidence to support the role of the β2 subunit in smoking cessation in humans. rs2072661 in the *CHRNB2* gene has been associated with smoking cessation success and time to relapse in a nAChR systems-based analysis of data from a bupropion placebo-controlled randomized clinical trial (Conti et al. 2008). Smokers who carry the minor allele had lower abstinence rates and more severe withdrawal severity than those heterozygous for wild-type allele (Conti et al. 2008). Perkins et al. (2009) examined the association of this polymorphism with ability to quit during a week of nicotine versus placebo patch use. Consistent with the prior study (Conti et al. 2008), smokers who carried the minor allele were less successful maintaining abstinence on NRT versus placebo compared to those with the wild-type genotype (Perkins et al. 2009). However, the function of this specific variant is unknown. More recently, King et al. (2011) reported *CHRNB2* SNPs rs3811450 and rs4262952 were associated with abstinence in smokers treated with varenicline. There is also evidence that *CHRNB2* interacts with *CHRNA4* in affecting nicotine dependence (Li et al. 2008). Thus, future studies of smoking cessation that are sufficiently powered to detect gene–gene interactions could extend this finding.

It is apparent from the animal and human studies which reviewed that *CHRNB2* is an important gene in maintaining nicotine dependence and contributes to significant symptoms of nicotine withdrawal. Smoking cessation therapies targeted to individuals with specific alterations in this gene may provide relief from the affective symptoms that promote relapse and continued use. However, additional research is necessary to provide the evidence base for this clinical approach.

CHRNA7

α7 nACh receptors are homomeric, comprising of five α7 subunits. Along with α4β2* nAChRs, α7 nAChRs are the most widely expressed nAChRs in the central nervous system (Dani and De Biasi 2001; Hogg et al. 2003). α7 nAChRs are activated by acetylcholine and blocked by αBgtx with high calcium permeability and a rapid desensitization rate (Gotti and Clementi 2004). Presynaptic α7 nAChRs facilitate neurotransmitter release (Alkondon et al. 2000) and have been implicated in several effects of nicotine, including aversion and reward (Laviolette and van der Kooy 2003), anxiety-like behavior (Tucci et al. 2003), and working memory (Levin 2002). α7 KO mice have impaired attentional performance (Hoyle et al. 2006; Young et al. 2007) and α7 antagonists impair spatial working memory (Levin et al. 2003), while α7 agonists significantly improve learning, memory, and attentional function in rodents (Bitner et al. 2007; Boess et al. 2007; Levin et al. 1999), and reverse aging-induced cognitive impairments (Beracochea et al. 2008; Marighetto et al. 2008), but there is less evidence for the role of α7 nAChRs in nicotine reinforcement (Smith et al. 2007).

The role of the α7 subunit, encoded by the *CHRNA7* gene, in nicotine dependence or withdrawal is not fully understood, and studies have found few differences in the effects of nicotine between α7 KO and wild-type mice (Davis and Gould 2007; Walters et al. 2006). Damaj et al. (2003) found that α7 nAChRs contribute to some nicotine withdrawal signs, but Grabus et al. (2005) and Markou and Paterson (2001) found that methyllycaconitine (MLA), an α7 nAChR antagonist, did not precipitate withdrawal.

α7 KO mice have been widely studied to explore the role of this subunit in nicotine's effects. Grabus et al. (2005) examined withdrawal after chronic oral nicotine administration in α7 KO mice by replacing nicotine solutions with water. Withdrawal-induced hyperalgesia

was attenuated in α7 KO compared to wild-type mice, but there was no difference in other aspects of nicotine withdrawal, such as the somatic signs (paw tremors, backing, head shakes). Similarly, Jackson et al. (2008) found that mecamylamine (MEC)-precipitated nicotine withdrawal in α7 KO mice implanted with osmotic mini-pumps resulted in a loss of hyperalgesia. No differences in the somatic signs of withdrawal were observed between nicotine-dependent wild-type α7 KO mice compared to saline-treated wild-type and α7 KO mice. In contrast, using a nicotine withdrawal protocol similar to Jackson et al. (2008) mice lacking the α7 subunit exhibited decreases in the somatic signs of MEC-precipitated withdrawal and exhibited normal MLA-precip-itated nicotine withdrawal, compared to wild-type mice (Salas et al. 2007). The opposing results from Jackson et al. (2008) and Salas et al. (2007) indicate a clearly undefined role of the α7 subunit in nicotine withdrawal, suggesting the possibility that there may be α7* nACh receptors that assemble with other subunits (Khiroug et al. 2002; Palma et al. 1999) that respond differently to the nAChR antagonists used to precipitate withdrawal. MEC is highly selective for α3β4* nAChRs, has a very low affinity for α7 nAChRs (Papke et al. 2001), and has been shown to be the most effective nAChR antagonist at precipitating both the somatic and non-somatic signs of withdrawal (Damaj et al. 2003; David et al. 2007; Malin et al. 1998). MLA, however, is a selective antagonist for α7 nAChRs (Roegge and Levin 2006) and has demonstrated contrasting results in nicotine withdrawal. MLA did not induce somatic signs of withdrawal after prolonged nicotine exposure in rats (Markou and Paterson 2001), but consistent with Salas et al. (2007), Damaj et al. (2003) showed that MLA was able to precipitate withdrawal, suggesting that MLA may have specificity for nAChR subunits other than α7 and the observed symptoms of withdrawal may only partially due to the α7 subunit.

There are very few human genetic studies examining the role of the *CHRNA7* gene with smoking behavior phenotypes. The results from a study of Jewish females indicate that *CHRNA7* SNPs are associated with severity of nicotine dependence in females (Greenbaum et al. 2006) but there is no known association of *CHRNA7* with smoking cessation (Conti et al. 2008; Ray et al. 2010). In a systems-based genetic study of smoking cessation, 1,295 SNPs in 58 genes within the neuronal nAChR and dopamine systems were investigated for their role in cessation within a bupropion placebo-controlled randomized clinical trial (Conti et al. 2008). A follow-up study on an independent sample of smokers treated with transdermal nicotine also assessed genes involved in acetylcholine synthesis and transport (Ray et al. 2010). Neither study identiWed significant SNPs in the *CHRNA7* gene that contributed to smoking cessation phenotypes. However, King et al. (2011) recently reported that, in treatment-seeking smokers randomized to varenicline, *CHRNA7* SNP rs6494212 was associated with abstinence.

Acetylcholine synthesis

Choline acetyltransferase (ChAT)

ChAT is responsible for the synthesis and modulation of endogenous acetylcholine (Whittaker 1988). ChAT influences several cholinergic-dependent functions including cognitive performance (Bacciottini et al. 2001) and has been shown to mediate modulatory effects cholinergic receptors on mesocorticolimbic dopaminergic pathways (Gronier et al. 2000). Chronic nicotine administration in adult rats has been shown to increase ChAT enzyme activity (Hernandez and Terry 2005) and ChAT enzyme activity is altered during nicotine withdrawal in adolescent animals (Slotkin et al. 2008). Molecules that alter ChAT enzyme activity have been tested clinically. Galantamine, an acetyl-cholinesterase inhibitor that increases the concentration of acetylcholine, may reduce smoking behavior (Diehl et al. 2006) suggesting that *ChAT* is a biologically plausible candidate gene for smoking behavior phenotypes.

In a sample of treatment-seeking smokers and an independent sample of family-based non-treatment seekers consisting of either African-Americans or European Americans, significant *ChAT* SNP and haplotype associations were identified for multiple measures of nicotine dependence (Ray et al. 2010; Wei et al. 2010). With respect to smoking cessation, Ray and colleagues performed a systems-based genetic association analysis in a discovery sample of 472 treatment-seeking smokers of European ancestry, assessing smoking relapse following 8 weeks of transdermal nicotine therapy. A cluster of SNPs in *ChAT* haplotype block 6 was significantly associated with relapse (Ray et al. 2010). These results are consistent with Heitjan et al. (2008) who found that *ChAT* SNP rs1917810 in haplotype 6 was associated with response to bupropion and smokers with the minor allele had higher abstinence rates on placebo.

To date, few human genetic association studies have examined the role of *ChAT* in smoking cessation. Future studies are needed for further characterization of *ChAT* and identiWcation of functional variants that may alter expression levels or enzymatic function. The initial findings of genetic associations of *ChAT* make *ChAT* an interesting candidate for future research.

Pharmacokinetics of nicotine

Cigarette smoking produces a rapid distribution of nicotine through the bloodstream and nicotine crosses the blood–brain barrier, with drug levels peaking in the brain within 10 s of inhalation (Le Houezec 2003). Smoking produces high concentrations of nicotine in the brain that are comparable to concentrations observed after intravenous administration (Hukkanen et al. 2005). Various studies have shown that smokers will try to maintain their plasma nicotine concentration within a narrow range by modulating their smoking behavior. This may include altering the number of cigarettes they smoke per day or how they smoke a cigarette (e.g., depth of inhalation) (Benowitz 2008).

The elimination half-life of nicotine is around 2 h. Among several nicotine metabolites, the major metabolite is cotinine. In humans, 70–80% of nicotine is converted to cotinine, mediated largely by the liver enzyme cytochrome P450 CYP2A6. Approximately 33–40% of cotinine is converted to its primary metabolite, 3′-hydroxycotinine (3HC), also by CYP2A6. Cotinine and 3′-hydroxycotinine are metabolized at a much slower rate than nicotine, and their rates of elimination are not significantly affected by changes in liver blood flow (Hukkanen et al. 2005; Mwenifumbo and Tyndale 2007).

Due to the slow rate of elimination, cotinine and its metabolite 3HC are useful in assessing nicotine exposure and metabolism. The ratio of 3HC to cotinine has been found to be a reliable marker for CYP2A6 activity and for individual differences in the rate of nicotine metabolism (Dempsey et al. 2004). Metabolism is faster in woman than in men, and reduced metabolism is found more frequently among Asians and African-Americans than among Caucasians and Hispanics (Benowitz et al. 2006a). Rate of nicotine metabolism has been associated with genetic polymorphisms in *CYP2A6* (Benowitz et al. 2006b; Bloom et al. 2011; Malaiyandi et al. 2005; Mwenifumbo and Tyndale 2007); however, twin studies suggest that known *CYP2A6* variants account for only a portion of the heritability of nicotine clearance rates (Ray et al. 2009; Swan et al. 2005).

CYP2A6

Several reduced and null activity alleles of the *CYP2A6* gene have been identified and shown to alter enzyme activity and in vivo nicotine metabolism (Fernandez-Salguero et al. 1995; Goodz and Tyndale 2002; Mwenifumbo et al. 2008; Mwenifumbo and Tyndale 2007). The *CYP2A6*1* allele is the “normal” activity (wild-type) variant and *CYP2A6*1B* is an

increased activity allele (faster nicotine clearance). The *CYP2A6*2* and *4 alleles are null activity alleles, occurring in about 20% of Asians and 1% of Caucasians (Nakajama et al. 2006; Rao et al. 2000; Schoedel et al. 2004). The *CYP2A6*9* and *12 are found more commonly in Caucasian populations, about 9% of Caucasians carry one or more of these alleles (Malaiyandi et al. 2005; Schoedel et al. 2004). These alleles have reduced activity (slower nicotine clearance) (Mwenifumbo et al. 2008; Mwenifumbo and Tyndale 2007; Ray et al. 2009).

Genetic variability in *CYP2A6* has been associated with a variety of smoking behavior phenotypes. Reduced or null activity *CYP2A6* alleles (*CYP2A6*9*, *CYP2A6*12*, *CYP2A6*2*, or *CYP2A6*4*) are more prevalent in non-smokers than smokers (Iwahashi et al. 2004; Malaiyandi et al. 2005; Mwenifumbo and Tyndale 2007; Pianezza et al. 1998). Smokers with reduced or null activity smoke fewer cigarettes (Audrain-McGovern et al. 2007; Fujieda et al. 2004; Malaiyandi et al. 2005, 2006; Minematsu et al. 2003; Mwenifumbo et al. 2007; Rao et al. 2000; Schoedel et al. 2004; Tyndale et al. 1999) and tend to be less dependent on nicotine (Kubota et al. 2006; Malaiyandi et al. 2006) than smokers with normal activity alleles. With respect to smoking initiation, adolescents with normal activity alleles may progress to nicotine dependence more quickly than slower metabolizers (*CYP2A6*9*, *CYP2A6*12*, *CYP2A6*2*, or *CYP2A6*4*) (Audrain-McGovern et al. 2007). A recent GWAS conducted in 31,266 smokers found an association of the rs4105144 SNP with reduced smoking quantity, assessed as cigarettes per day (Thorgeirsson et al. 2010). The rs4105144 SNP is in linkage disequilibrium with the *CYP2A6*2* reduced activity allele.

A few studies have reported associations of *CYP2A6* with smoking cessation and response to treatment. Smokers with the null activity allele, *CYP2A6*2*, are twice as likely to quit smoking as smokers who do not possess that allele (Gu et al. 2000). Further, smokers with high activity alleles (*CYP2A6*1*/*1B*) report more serious withdrawal symptoms during smoking cessation (Kubota et al. 2006). The correlation between *CYP2A6* genotypes and withdrawal symptoms was more notable in smokers who used NRT, such as transdermal nicotine patch in which a high dose of nicotine is administered initially with a gradual decrease in dosage. Smokers with high CYP2A6 activity, treated with NRT, might maintain a low level of nicotine that desensitizes/inactivates a larger number of receptors than smokers with low activity.

Nicotine metabolite ratio—Variation in nicotine metabolism rate can also be determined by the ratio of the nicotine metabolites (3-HC/cotinine) that is derived from cigarette smoking (Benowitz et al. 2003). The ratio of 3-HC/cotinine is generally referred to as the nicotine metabolite ratio and can be measured reliably in saliva or plasma (Dempsey et al. 2004) and is independent of smoking patterns or time since last cigarette, among regular smokers (Levi et al. 2007). Null or reduced activity *CYP2A6* alleles (*CYP2A6*2, *4, *9*, and *12*) are associated with lower nicotine metabolite ratios and slower metabolism (Dempsey et al. 2004; Johnstone et al. 2006; Malaiyandi et al. 2006). At present, use of the nicotine metabolite ratio may be more preferable to assessing *CYP2A6* genotype, given the large number of *CYP2A6* alleles and the presence of non-genetic influences on nicotine metabolism rates (Ray et al. 2009).

Lerman and colleagues assessed 480 treatment-seeking smokers who were randomized to 8 weeks of transdermal nicotine or nicotine nasal spray. The nicotine metabolism ratio predicted the effectiveness of transdermal nicotine treatment at the end of treatment and at 6-month follow-up; however, the nicotine metabolite ratio was not a predictor of abstinence with use of nicotine nasal spray, presumably because smokers were able to titrate the dose of nasal spray based on differential metabolism (Lerman et al. 2006). The nicotine metabolite ratio was validated as a predictor of quitting success in an independent sample of smokers

after 8 weeks of transdermal nicotine treatment (Schnoll et al. 2009). Based on these results, Lerman and others examined the efficacy of extended (6-month) transdermal nicotine therapy versus the standard 8-week therapy in 471 Caucasian smokers with either normal or reduced rates of nicotine metabolism. Reduced metabolizers were found to benefit more from extended therapy than standard 8-week therapy compared to normal metabolizers (Lerman et al. 2010).

To determine whether the predictive validity of the nicotine metabolite ratio is specific for transdermal nicotine therapy, another study evaluated this biomarker among 414 smokers in a 10-week placebo-controlled randomized trial of bupropion (Patterson et al. 2008). Faster metabolizers had significantly lower quit rates than slower metabolizers if treated with placebo, but had equivalent quit rates to slower metabolizers if treated with bupropion (Patterson et al. 2008). In a clinical trial of African-American light smokers, slower metabolizers based on the nicotine metabolite ratio had higher quit rates than faster metabolizers treated with nicotine gum or placebo (Ho et al. 2009).

Withdrawal symptoms interfere with smoking cessation (Shiffman et al. 2006) and faster metabolizers of nicotine may experience more intense withdrawal symptoms due to a more rapid decline in blood and brain nicotine concentrations after smoking a cigarette. In adolescent light smokers, faster metabolizers reported greater withdrawal symptoms after 24 h and described themselves as more highly addicted than slow metabolizers (Rubinstein et al. 2008). In adult treatment-seeking smokers, higher nicotine metabolite ratios predicted more severe cravings for cigarettes after 1 week of treatment with transdermal nicotine (Lerman et al. 2006); however, there was no significant association of the nicotine metabolite ratio with withdrawal symptoms or nicotine craving in a second study of transdermal nicotine (Schnoll et al. 2009) or in the bupropion placebo-controlled trial (Patterson et al. 2008). It is possible that differences in very early withdrawal symptoms exist, but were not captured by the measurement points used in the clinical trials.

CYP2B6

The role of the CYP2B6 enzyme in nicotine metabolism is unclear. There is some evidence for a small role in the metabolism of nicotine to cotinine (Dicke et al. 2005; Yamazaki et al. 1999). CYP2B6 may contribute to nicotine metabolism that is not mediated by CYP2A6 (Messina et al. 1997). The *CYP2B6*6* haplotype (Q172H and K262R variants) is associated with faster nicotine and cotinine clearance (Benowitz et al. 2006b; Ring et al. 2007) and the *CYP2B6*4* allele is associated with higher 3-HC/cotinine ratios (Johnstone et al. 2006) among individuals with reduced CYP2A6 activity.

The CYP2B6 enzyme is the primary enzyme involved in the metabolism of the bupropion (Faucette et al. 2000) and the *CYP2B6*5* and **6* variants are associated with slower bupropion metabolism (Hesse et al. 2004; Lang et al. 2001; Loboz et al. 2006). *CYP2B6* genotype was examined as a predictor of relapse in a placebo-controlled trial of bupropion for smoking cessation (Lerman et al. 2002). 426 participants of European ancestry were genotyped for the functional C1459T (*CYP2B6*5*) variant and received bupropion or placebo for 10 weeks. Female smokers carrying the decreased activity allele reported greater increases in cravings following the target quit date and had higher relapse rates on placebo, which were reversed with bupropion (Lerman et al. 2002). David and others examined a sample of 291 smokers of European ancestry who participated in a double-blind randomized 12-week trial of bupropion versus placebo. Individuals who carried both the *CYP2B6*5* allele and the dopamine D2 receptor *DRD2*-Taq1A allele demonstrated the highest long-term abstinence rates with bupropion (David et al. 2007).

The *CYP2B6*6* haplotype is defined as having both the Q172H and K262R variants and is the most common *CYP2B6* haplotype (Lee et al. 2007). Smokers carrying the *CYP2B6*6* genotype exhibited greater quitting success with bupropion compared to placebo at the end of treatment and 6-month follow-up. However, the genotype by treatment interaction was driven by differential quit rates by genotype on placebo; the two genotype groups had equivalent quit rates on bupropion suggesting that this association may be mediated by nicotine metabolism rather than bupropion metabolism (Lee et al. 2007). Recently, several *CYP2B6* polymorphisms were associated with abstinence among treatment-seeking smokers randomized to bupropion therapy (King et al. 2011), although their function is not known.

The reviewed studies suggest that individual variability in the rate of nicotine metabolism influences smoking behavior and response to smoking cessation treatments. Given the large number of *CYP2A6* alleles, as well as the influence of environmental factors on nicotine metabolism, a phenotypic biomarker of CYP2A6 activity (3HC/cotinine) appears to be a more robust predictor of cessation than genotype.

Conclusions

This review examined the evidence for the association of cholinergic and nicotine metabolism genes with smoking cessation and therapeutic response. Although SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster have reproducible associations with smoking heaviness, evidence supporting the role of the *CHRNA5-CHRNA3-CHRNB4* gene cluster in smoking cessation is mixed. There is initial evidence for the role of the *CHRNB2* gene in smoking cessation. Smokers who carry the minor allele of rs2072661 had lower abstinence rates in two independent trials; however, the function of this *CHRNB2* variant is unknown (Conti et al. 2008; Perkins et al. 2009). Human genetic studies examining the *ChAT* gene have also reported initial associations with smoking cessation (Heitjan et al. 2008; Ray et al. 2010). The function of the SNPs in *ChAT* is unknown and further evidence of replication is important. There is no replicated evidence yet supporting a role of *CHRNA6-CHRNB3*, *CHRNA4*, and *CHNRA7* genes with smoking cessation as yet.

The research on genetic variation in nicotine metabolism demonstrates reproducible and clinically significant effects of nicotine metabolism rate on smoking cessation. Smokers with reduced or null activity alleles of *CYP2A6* metabolize nicotine more slowly, and may experience less severe withdrawal symptoms and be more likely quit smoking (Gu et al. 2000; Kubota et al. 2006). The nicotine metabolite ratio is a reliable phenotypic measure to examine the rate of nicotine metabolism in smokers (Ray et al. 2009). Five independent studies show significant associations of this pre-treatment marker with smoking cessation and response to pharmaco-therapy (Ho et al. 2009; Lerman et al. 2010; Lerman et al. 2006; Patterson et al. 2008; Schnoll et al. 2009). There is also some support for *CYP2B6* genotypes predicting smokers' response to bupropion treatment for smoking cessation (David et al. 2007; Lee et al. 2007; Lerman et al. 2002).

Despite initial encouraging data, there are several limitations of the smoking cessation genetic studies conducted to date. Many of the clinical trials have relatively small sample sizes (300–500 subjects). A large portion of the smoking population, such as ethnic minorities, are not accounted for in these studies. Larger sample sizes and studies that target various ethnic groups may help to identify novel genetic associations with smoking cessation. Another limitation is that the function of many of the gene variants examined is still unknown. At least for the genotype markers, eVect sizes tend to be small and the studies are not powered to detect gene–gene and gene–environment interactions important in cessation.

It will be important to understand the mechanistic basis of the association of genetic variants with smoking cessation. Nicotinic receptors are involved in many neuronal functions, including differentiation and synaptic plasticity, which are the basis for learning and memory. Response inhibition, attention, and working memory are influenced directly by nAChRs or through nAChR interactions with other neurotransmitter systems (Rezvani and Levin 2001). The cognitive signs of nicotine withdrawal are believed to be an important factor in failed cessation attempts and continued use (Koob et al. 1993; Markou et al. 1998; Patterson et al. 2010). The nAChR *CHRNA4* SNP rs1044396 contributes to individual differences in visuospatial attention (Greenwood et al. 2005) and another study showed a strong association of rs6090384 with severe inattention (Todd et al. 2003). Recent tobacco use improved cognitive flexibility in European American smokers with variants in the *CHRNA5-CHRNA3-CHRNB4* gene cluster, implying that nicotine can enhance various aspects of cognitive processing by activation of nicotinic receptors and may modulate the effect of genetic factors on cognitive flexibility (Marchant et al. 2008; Zhang et al. 2010)

Overall, there is still much unknown about the role of genetic variability in cholinergic and nicotine metabolism genes in smoking cessation. Research examining the cellular and molecular mechanisms of SNPs and haplotypes in genes associated with smoking cessation phenotypes can facilitate the development of novel pharmacotherapies and the delivery of personalized smoking cessation treatment.

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