Trends in Pharmacological Sciences

CellPress

Special Issue: Precision Medicine

Review

Pharmacogenetic Optimization of Smoking Cessation Treatment

Meghan J. Chenoweth[1,2] and Rachel F. Tyndale[1,2,3,*]

Worldwide, approximately one billion people smoke cigarettes. Cigarette smoking persists in part because long-term smoking cessation rates are modest on existing treatments. Smoking cessation outcomes are influenced by genetic factors, including genetic variation in enzymes that metabolize nicotine and smoking cessation medications, as well as in receptor targets for nicotine and treatment medications. For example, smokers with genetically slow nicotine metabolism have higher cessation success on behavioural counseling and nicotine patches compared with smokers with genetically fast nicotine metabolism. In this review, we highlight new progress in our understanding of how genetic variation in the pharmacological targets of nicotine and smoking cessation medications could be used to tailor smoking cessation therapy, increase quit rates, and reduce tobacco-related harm.

Trends

Slower (versus faster) nicotine metabolizers, by *CYP2A6* genotype or CYP2A6 phenotype (the nicotine metabolite ratio; NMR), have higher smoking abstinence rates on behavioural counseling, nicotine patches, and in the absence of treatment ('cold turkey')

In smokers assigned to treatment based on their NMR, slower metabolizers had similar quit rates on varenicline and nicotine patches, while faster metabolizers quit better on varenicline (versus nicotine patches)

In smokers with genetically slow (versus normal) *CYP2B6* activity, which metabolically activates bupropion, smoking cessation rates were lower on bupropion and placebo

Genetic variation in the central nervous system targets of nicotine (e.g., nicotinic receptors and dopaminergic pathways) has been associated with smoking cessation, albeit inconsistently

Applying Pharmacogenetics to Reduce Cigarette Smoking

Tobacco smoking persists despite decades of research demonstrating its harm to health and the availability of three types of pharmacotherapy for treating tobacco dependence [nicotine replacement therapy (NRT); bupropion, and varenicline], which bind selected subtypes of nicotinic acetylcholine receptors (nAChRs) to exert their varying effects [1–3]. Bupropion can additionally bind to the dopamine transporter, which is thought to also contribute to its pharmacological action [4]. A large proportion of the ability to quit smoking is heritable (~50–60%) [5]. During the late 1990s, genetic variation in the major pathway of nicotine metabolism was shown to alter the quantity of cigarettes smoked [6]. In more recent years, genetic variation in nicotine metabolism and receptor genes, and in dopaminergic pathway genes, has been implicated in the ability to quit smoking both in the absence and presence of pharmacotherapy. Genetic factors with more general effects on cessation are likely to influence both shorter (e.g., 8-weeks) and longer (e.g., 6-months) term abstinence, while those that influence cessation on pharmacotherapy are likely to be more apparent during the period of active treatment, but can have lasting effects. Recently, several meta-analyses have indicated the robustness of some of these specific genetic findings [7,8]. Single-gene and polygenic influences on smoking cessation, within and between treatment conditions, have been studied and are reviewed here; the identification of gene variants associated with higher quit rates on one treatment relative to another will likely be especially valuable in clinical settings for personalizing treatment. Genomics-based personalization of tobacco dependence treatment will, in turn, increase smoking cessation rates and reduce the burden of tobacco-related disease.

[1]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ONT, Canada
[2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ONT, Canada
[3]Department of Psychiatry, University of Toronto, Toronto, ONT, Canada

*Correspondence: r.tyndale@utoronto.ca (R.F. Tyndale).

Optimizing Cessation Using Genetics of Drug Metabolism Enzymes

The Metabolic Pathways of Nicotine

Nicotine is the major psychoactive compound in cigarette smoke [9]. The rate at which nicotine is metabolically inactivated, or cleared, from the body has important implications for nicotine dependence and the ability to quit smoking. Up to 80% of a nicotine dose is metabolically inactivated to cotinine, principally (~90%) by the hepatic enzyme cytochrome P450 (CYP) 2A6 [10] (Figure 1), with minor contributions from CYP2B6 (10%) [11]. Nicotine is also metabolized to more minor metabolites by additional enzymes, including FMO3 and UGT2B10 [12]. The majority of cotinine undergoes further metabolism to 3′-hydroxycotinine in a reaction exclusively mediated by CYP2A6 [13] (Figure 1). The 3′-hydroxycotinine:cotinine ratio, known as the

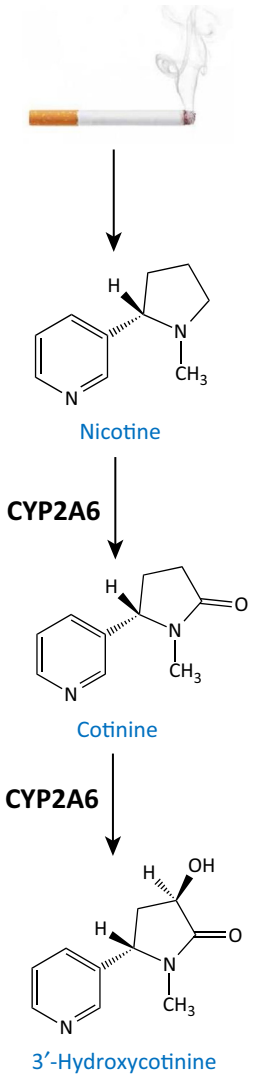

Figure 1. Role of Cytochrome P450 (CYP)-2A6 in the Metabolic Pathway of Nicotine. Up to 80% of a nicotine dose inhaled from cigarette smoke undergoes metabolic inactivation to cotinine. CYP2A6 is responsible for approximately 90% of the inactivation of nicotine to cotinine. Most cotinine is then further metabolized to 3′-hydroxycotinine, in a reaction mediated wholly by CYP2A6. The ratio of these two metabolites (3′-hydroxycotinine and cotinine), known as the nicotine metabolite ratio or NMR, serves as a biomarker of CYP2A6 activity. Faster CYP2A6 activity is reflected by a higher NMR.

nicotine metabolite ratio (NMR), is an established and validated phenotypic indicator of CYP2A6 activity in daily smokers; faster CYP2A6 activity is reflected by a higher NMR [14–18].

CYP2A6 Genetics and Smoking Cessation Outcomes

The *CYP2A6* gene is highly polymorphic, with many variants altering CYP2A6 function; individuals can be genotyped for these variants and grouped into *CYP2A6* activity groups (e.g., faster and slower metabolizers) based on the predicted metabolic impact of their *CYP2A6* genotype on nicotine clearance [19]. In addition to capturing the influence of any existing *CYP2A6* polymorphisms, the NMR also reflects environmental sources of variation in CYP2A6 activity (e.g., use of estrogen-containing hormonal therapy [20]). Similar to genotype-based activity groupings, smokers can be dichotomized as faster and slower metabolizers based on NMR. There is currently no single optimal NMR cut-point used to distinguish slower from faster metabolizers for cessation optimization or other smoking phenotypes (see Outstanding Questions). Different investigations have selected NMR cut-points based on sensitivity and specificity analyses of smoking cessation outcomes and/or pragmatic reasons (e.g., recruitment goals or statistical power); across different studies, slow metabolizers generally represent the lowest 25–40% of the NMR distribution [21,22]. Slower nicotine metabolizers (determined by *CYP2A6* activity group or NMR) have lower cigarette consumption [23], dependence [24,25], nAChR availability [26], and brain response to smoking (versus control) cues [27,28], compared with faster nicotine metabolizers. Slower nicotine metabolizers also display higher smoking cessation rates in the absence of pharmacotherapy [29–31].

In addition to NMR, an alternative *CYP2A6* genotype-based measure of nicotine metabolism rate exists that uses the ratio of deuterated (D_2) cotinine/(D_2cotinine + D_2nicotine) determined 30 min after the oral administration of D_2 nicotine [32]. In Caucasian treatment-seeking smokers randomized to placebo, those within the predicted lowest quartile of nicotine metabolism rate had a lower relapse risk versus faster metabolizers (hazard ratio = 0.40; $P = 0.013$). Similarly, on placebo, Caucasian smokers with slower rates of CYP2A6 activity (lower NMR) were less likely to relapse than were those with faster rates [31]. Variation in NMR is also associated with smoking cessation outcomes on active pharmacotherapy, as discussed below.

The Nicotine Metabolite Ratio and Smoking Cessation Outcomes

In smokers randomized to nicotine patch therapy, slower nicotine metabolizers (lowest NMR quartile) displayed higher quit rates compared with faster metabolizers [33,34]. In a separate trial, smokers within the lowest NMR quartile had higher quit rates on extended (6-month) versus standard (8-week) nicotine patch therapy; by contrast, those with faster rates of nicotine metabolism did not benefit from extended therapy [35]. In another small trial of faster nicotine metabolizers (NMR $\geq$ 0.18), there was a trend toward higher end-of-treatment quit rates on 42-mg patches versus 21-mg patches (46% versus 30%; $P = 0.08$) [21]. Thus, the nicotine patch, at higher doses, may be effective in those with higher NMR. Follow-up studies are required to determine whether these findings replicate beyond clinical trial settings. In community-based smokers receiving nicotine patch therapy, faster metabolizers (NMR $\geq$ 0.47) displayed lower end-of-treatment quit rates compared with slower metabolizers (24% versus 33%; $P = 0.03$) [36]. To our knowledge, this is the first study demonstrating that relations between NMR and cessation outcomes replicate in community-based smokers.

In smokers prospectively randomized to treatment based on NMR, varenicline was more efficacious than were nicotine patches in faster metabolizers (NMR $\geq$ 0.31) as hypothesized; in slower metabolizers, quitting did not differ by treatment [22] (Figure 2A, Key Figure). In faster metabolizers, the number needed to treat (NNT) for patches (versus placebo) and varenicline (versus placebo) was 26 and five, respectively; for slower metabolizers, the NNT values were ten and eight, respectively [22] (Figure 2B). Moreover, varenicline (versus placebo) was associated

Key Figure

The Nicotine Metabolite Ratio (NMR) Influences Smoking Cessation Outcomes on Varenicline and Nicotine Patches

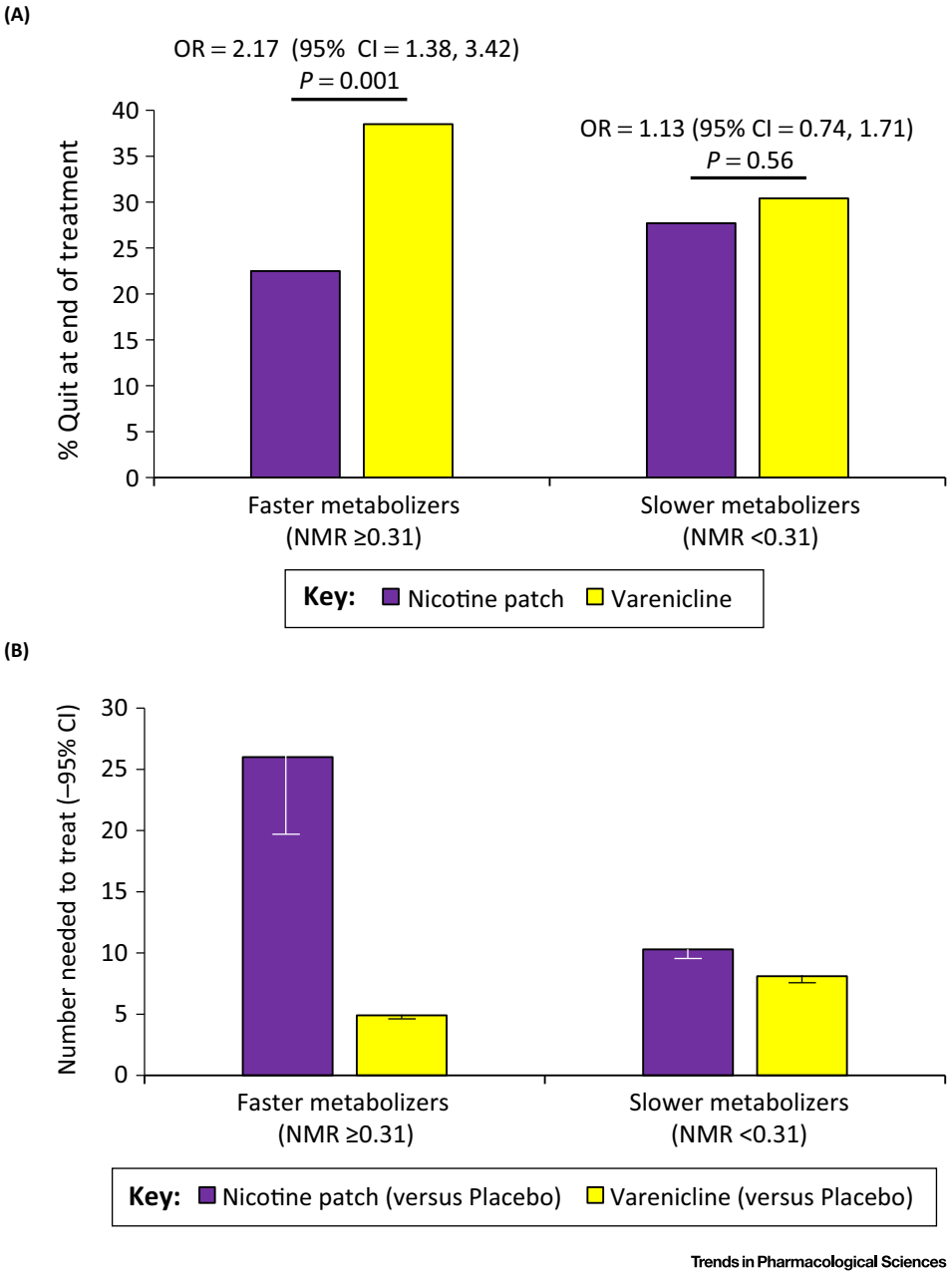

Figure 2. In smokers prospectively randomized to varenicline, nicotine patches, or placebo based on the NMR, end-of-treatment quit rates and odds ratios (OR) with 95% confidence intervals (CI) comparing the efficacy of varenicline versus the nicotine patch as a function of NMR group (faster versus slower nicotine metabolizers) are shown in (A). There was a significant NMR-by-treatment interaction on end-of-treatment quit rates: ratio of OR (ORR) = 1.89 (95% CI = 1.02, 3.45; $P = 0.04$) (A). In a longitudinal model including end-of-treatment as well as 6-month and 12-month quit rates, the NMR-by-treatment interaction was also significant: ORR = 1.96 (95% CI = 1.11, 3.46; $P = 0.02$). Number needed to treat analyses

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with greater adverse effect severity in slower (versus faster) metabolizers. By contrast, the nicotine patch (versus placebo) was well tolerated in both groups. These findings suggest that varenicline is more suitable for faster metabolizers, whereas the patch is more suitable for slower metabolizers [22]. Follow-up studies in community-based smokers evaluating NMR and comparing cessation outcomes on varenicline and patches will help inform the implementation of NMR as a clinical tool to personalize smoking cessation therapy.

CYP2B6 Genetics and Bupropion Treatment Outcomes

Like *CYP2A6*, *CYP2B6* is also highly polymorphic. The CYP2B6 protein is expressed in the liver and in extrahepatic tissues, including the brain, and metabolizes bupropion to its pharmacologically active metabolite hydroxybupropion [37]. Thus, variation in CYP2B6 activity that alters hydroxybupropion levels could influence bupropion-assisted cessation. Variability in the activity of CYP2A6, which does not metabolize bupropion, was not associated with bupropion-assisted cessation [31,32].

The common *CYP2B6*6* haplotype (e.g., ~25% in Caucasians [38]) comprises the *CYP2B6*4* (rs2279343) and *CYP2B6*9* (rs3745274) nonsynonymous variants, and is associated with lower hepatic CYP2B6 protein expression [11] and reduced metabolism of bupropion [39]. In Caucasian smokers, end-of-treatment quit rates on bupropion did not differ between individuals with no copies of *CYP2B6*6* versus those with one to two copies of *CYP2B6*6* (31% versus 33%, respectively; $P = 0.84$). However, bupropion and hydroxybupropion levels were not assessed in this study; thus, it is not possible to determine treatment adherence [40]. In verified treatment-adherent African-American light smokers (fewer than ten cigarettes/day) randomized to bupropion, slow *CYP2B6* metabolizers (including those with the *CYP2B6*6* haplotype) had lower plasma hydroxybupropion levels versus normal metabolizers; lower hydroxybupropion levels, in turn, were associated with poorer bupropion-assisted cessation [41]. In this study, *CYP2B6* genotype was not associated with bupropion adverse effects. Together with the efficacy data, these findings suggest that genetically slow *CYP2B6* metabolizers would benefit from a higher bupropion dose [41].

In a separate study of bupropion-treated Caucasian smokers, several additional *CYP2B6* variants were associated with cessation [42]. The G allele of rs8109525, present in a noncoding region ~5 kb upstream of *CYP2B6*, was associated with a higher likelihood of continuous abstinence at weeks 9–12 (OR = 1.8; $P = 0.0008$). This single nucleotide polymorphism (SNP) is not in linkage disequilibrium (i.e., not inherited together) with key *CYP2A6* functional polymorphisms and has been linked to lower CYP2B6 mRNA expression in human liver samples [43]. Thus, the *CYP2B6* rs8109525 G allele may be associated with lower CYP2B6 activity and bupropion metabolism to hydroxybupropion; the mechanism behind its association with higher bupropion-assisted cessation remains to be determined.

In addition to its role in bupropion metabolism, CYP2B6 is hypothesized to have a role in the central (brain) metabolism of nicotine. In rats, the selective inhibition of brain CYP2B, which is thought to mimic genetically slow *CYP2B6* metabolism in humans, was associated with higher brain (but not plasma) nicotine levels and a greater number of sessions required to extinguish nicotine self-administration behavior [44]. In placebo-treated Caucasian heavy smokers, those with one or two copies of *CYP2B6*6* had lower end-of-treatment quit rates versus those with no

comparing the effectiveness of varenicline versus placebo, and nicotine patches versus placebo, as a function of NMR group (faster versus slower nicotine metabolizers) is shown in (B). For example, 26 faster metabolizers would need to be treated with nicotine patches to have one smoker quit. The full results from this clinical trial (NCT01314001) are detailed in [22]. Abbreviation: CI, confidence interval.

copies of *CYP2B6*6* (14% versus 32%, respectively; $P = 0.01$) [40]. Thus, it appears that slow CYP2B6 activity may be associated with increased relapse risk on placebo [40] and possibly bupropion [41].

Genetic variation in CYP2C19, which can also metabolize bupropion [45], has also been investigated for possible involvement in bupropion-assisted cessation [46]. *CYP2C19*2* (rs4244285) was associated with higher bupropion area under the plasma concentration-time curve (AUC), but not the hydroxybupropion AUC, in 42 healthy volunteers. Consistent with its lack of association with hydroxybupropion AUC, there was no association between *CYP2C19*2* and cessation outcomes in bupropion-treated African-American smokers [46]. Thus, *CYP2C19* variation may not substantially influence bupropion-assisted cessation.

OCT2 Genetics and Varenicline Treatment Outcomes

Varenicline is the most effective smoking cessation drug, with odds ratios (ORs) of around three versus placebo and around two versus bupropion or NRT for ≥6-month abstinence [47]. Varenicline undergoes almost no metabolism [48] and, therefore, is unlikely to be influenced by polymorphisms in drug-metabolizing enzymes. However, variation in the transport of varenicline throughout the body may alter varenicline pharmacokinetics and cessation outcomes. Varenicline is transported by organic cation transporter 2 (OCT2) [49], which is expressed in renal proximal tubule cells and endothelial cells of the blood–brain barrier [50]. The rs316006 T allele in the *OCT2* gene (*SLC22A2*) was associated with greater smoking abstinence at end-of-treatment and at 6 months in varenicline-treated Caucasian smokers [51]. Whether *OCT2* variation represents a useful target for the optimization of smoking cessation treatment remains to be determined.

Optimizing Cessation Using Genetics of Central Nervous System Targets

The reinforcing effects of nicotine are thought to result from its actions in the mesolimbic dopaminergic system in the brain. Nicotine binds nAChRs in the ventral tegmental area, which contains the cell bodies of the ventral mesolimbic pathway, evoking dopamine release in the nucleus accumbens [52]. Genetic variation in the targets of nicotine within the central nervous system, including nAChRs and components of the dopaminergic system (e.g., dopamine receptors and transporters), is associated with differences in smoking cessation. Nicotine replacement therapies, including patches, gum, and nasal sprays, which deliver nicotine more slowly than via smoking, also activate nAChRs and evoke dopamine release [1,53]. Similar to nicotine, bupropion also binds nAChRs; however, it has antagonistic activity at multiple nAChR subtypes [54]. Bupropion can also bind the dopamine transporter [4]. While nicotine has full agonist activity at $\alpha4\beta2$-containing nAChRs, varenicline is a partial agonist of this receptor subtype, but has higher relative binding affinity [55].

nAChRs

Variation in nAChR genes, particularly the *CHRNA5-CHRNA3-CHRNB4* cluster located on chromosome 15q25, has been examined for associations with smoking cessation success in the absence of treatment and with active pharmacotherapy. While the *CHRNA5-CHRNA3-CHRNB4* cluster is robustly associated with small differences in cigarette consumption and nicotine dependence [56], the associations between this cluster and cessation outcomes have often differed between studies. Of the SNPs investigated within this cluster, rs16969968, located in *CHRNA5*, has been studied most frequently.

nAChR Gene Variation and Smoking Cessation: Nontreatment-Seeking Smokers

In a meta-analysis of 24 studies in nontreatment-seeking Caucasian smokers, those with the *CHRNA5* rs16969968 AA risk genotype quit a median of 4 years later compared with GG smokers [8]. In a separate analysis of community-based Caucasian smokers, a high-risk haplotype defined by rs16969968 (A allele) and rs680244 (C allele) delayed smoking cessation

(self-reported) by a median of 2 years compared with lower-risk groups [57]. The *CHRNA5* rs16969968 A allele, which has also been associated with higher cigarette consumption and cotinine levels (a biomarker of tobacco exposure) [58], is thought to lead to a lower maximal nAChR response to nicotine [59]. In mice, *CHRNA5* gene knockout led to greater levels of nicotine intake [60]. Together, these data suggest that reduced *CHRNA5* activity increases the heaviness of smoking and decreases cessation.

nAChR Gene Variation and Smoking Cessation: Treatment-Seeking Smokers

In addition to delaying cessation in nontreatment-seeking smokers, the high-risk haplotype defined by rs16969968 (A allele) and rs680244 (C allele) was associated with relapse on placebo, but not active treatment, in an NRT and bupropion clinical trial in Caucasian smokers [57]. Similarly, in a separate study, the risk allele for rs1051730 (in near-perfect linkage disequilibrium with rs16969968) increased relapse on placebo [61]. Together, these data suggest that pharmacotherapy would blunt relapse risk in smokers with the high-risk haplotype [57]. However, the association between rs16969968 variation and relapse on placebo has not been replicated in all trials [62,63].

In contrast to unaided quitting or cessation on placebo [8,57,61], variation in rs16969968 does not appear to be associated with quitting on active pharmacotherapy. A recent meta-analysis in smokers (>95% Caucasian) receiving NRT showed no associations between rs16969968 or rs1051730 and end-of-treatment or 6-month quit rates [7] (Figure 3). This suggests that the influence of high-risk rs16969968/rs1051730 genotypes on increasing relapse [8,57,61] is mitigated by treatment with NRT.

In smokers receiving varenicline, *CHRNA5* rs16969968 was not associated with cessation [62], suggesting, as for NRT, that varenicline reverses the elevated relapse risk associated with the rs16969968 AA genotype. In Caucasian smokers prospectively randomized to nicotine patches or varenicline based on NMR [22], no associations for rs16969968 with end-of-treatment quit rates on nicotine patches or varenicline were observed [63]. Two other SNPs tagging loci within the *CHRNA5-CHRNA3-CHRNB4* cluster (rs588765 and rs578776) that were previously

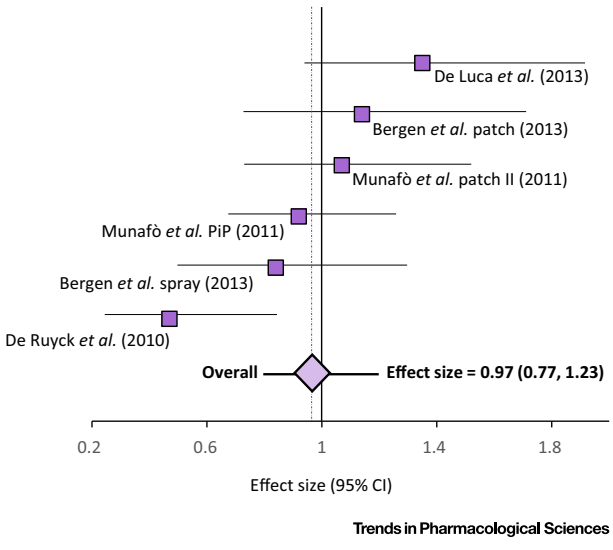

Figure 3. The rs1051730-rs16969968 Genetic Variant in the Nicotinic Receptor Does Not Influence Smoking Cessation Outcomes on Nicotine Replacement Therapy (NRT). Effect sizes (odds ratios) from four clinical studies [61,79–81] comprising NRT treatment arms show the likelihood of successful smoking cessation at end-of-treatment for smokers with the rs1051730-rs16969968 risk allele (rs1051730 T allele or rs16969968 A allele) relative to smokers with the rs1051730-rs16969968 reference allele (rs1051730 C or rs16969968 G allele). The overall effect size from the meta-analyzed data is also depicted. Results were generated from additive genetic models adjusting for age and sex. No significant heterogeneity was present in the meta-analysis ($I^2 = 50.6\%$; $P = 0.07$). The broken vertical line represents the overall effect size, which was 0.97. Abbreviations: CI, confidence interval; PiP, Patch in Practice study. Adapted from [7].

robustly associated with minor influences on cigarette consumption and dependence in Caucasians were also not associated with cessation outcomes in any arm [63]. Three separate placebo-controlled trials also found no association for rs16969968 with abstinence on varenicline during the last 4 weeks of treatment [42]. In Caucasian smokers receiving both nicotine patches and bupropion, there was also no association between rs16969968 and cessation; however, an alternative *CHRNA5* SNP, rs680244, was associated with abstinence at 52 weeks in those that received bupropion and nicotine patches in combination [64]. Together with the findings from untreated and placebo-treated smokers [8,57,61], these data suggest that pretreatment determination of rs16969968 genotype is useful for identifying subsets of smokers who are more likely to benefit from active pharmacotherapy versus behavioral counseling or no cessation interventions. The effects of treatment across rs16969968 genotype groups could be explicitly tested prospectively in a rs16969968-stratified clinical trial, similar to the approach used in the NMR-stratified trial [22], which may improve clinical recommendations for treating tobacco dependence.

In African-American light smokers, neither rs16969968 nor rs578776 was associated with smoking abstinence on active treatment (nicotine gum or bupropion) or placebo [65]. In the placebo-controlled nicotine gum trial, smokers with the rs588765 T allele had higher quit rates on nicotine gum; however, in those receiving placebo gum, the rs588765 T allele was associated with lower abstinence. Similar magnitudes and directions of effect for active versus placebo arms were observed in the bupropion trial. The A allele of another SNP, rs2036527, located ~6.2 kb 5′ of *CHRNA5*, was associated with lower quit rates on nicotine gum and bupropion during and at end-of-treatment (unadjusted ORs = 0.31–0.59). However, in the placebo arms of both trials, there was no association between rs2036527 and abstinence [65].

Beyond the *CHRNA5-CHRNA3-CHRNB4* cluster, genetic variation in other nAChR subtypes has been associated with smoking cessation in Caucasians. The *CHRNB2* rs2072661 A allele was associated with lower quitting in both bupropion and placebo-treated smokers [66]. Analyses in three separate placebo-controlled clinical trials identified additional *CHRNB2* SNPs (rs3811450 and rs4292956), as well as SNPs in other nAChR subunits, including *CHRNA4* (rs3787138 and rs2236196) and *CHRNA7* (rs6494212), which influenced abstinence on varenicline [42]. Whether these findings will replicate in other varenicline-treated smokers or extend to other treatments remains to be determined.

Together, the lack of replicated findings for nAChR gene variants and smoking cessation outcomes reduces the likelihood that this genomic region will be useful in the personalization of smoking cessation therapy. Comparing cessation outcomes on active pharmacotherapy with a single, well-selected placebo group may facilitate the interpretation of findings from such studies [62].

Dopaminergic Pathway Gene Variation and Smoking Cessation

Genetic variation in components of the dopaminergic system has also been investigated as a potential source of variability in smoking cessation rates. In general, functional polymorphisms that lead to lower dopaminergic tone (i.e., reduced dopaminergic activity) are thought to contribute to lower smoking cessation success [67].

Variation in the dopamine D4 receptor gene (*DRD4*) has been examined in placebo-controlled clinical trials. The exon-III VNTR polymorphism in *DRD4* was not associated with overall abstinence in Caucasian smokers receiving placebo or bupropion [68]. However, in smokers with one or more copies of the long allele (seven or more repeats), which may lead to lower DRD4 mRNA expression [69], bupropion increased cessation (versus placebo); smokers with two copies of the short allele (fewer than seven repeats) did not benefit from bupropion [68]. In a

separate sample of Caucasian smokers, Bergen and colleagues similarly observed a larger, albeit nonsignificant, benefit of bupropion (versus placebo) in those with the long allele (versus those homozygous for the short allele) [70]. Together, these data suggest that bupropion is a more suitable treatment for smokers with the *DRD4* exon-III VNTR long allele. In a placebo-controlled nicotine patch trial in Caucasian smokers, there was an overall association for the *DRD4* exon-III VNTR long allele with reduced cessation at end-of-treatment (12 weeks); however, the effect of nicotine patches (versus placebo) on cessation did not differ as a function of *DRD4* genotype, suggesting that nicotine patch therapy worked equally well in both groups of smokers [67]. These latter findings do not support the use of this *DRD4* polymorphism in tailoring cessation treatment with nicotine patches.

Polymorphisms associated with the dopamine transporter (*SLC6A3*) and dopamine D2 receptor (*DRD2*) genes have also been examined as potential modulators of smoking cessation outcomes. At end-of-treatment, neither the 3′ VNTR polymorphism in *SLC6A3* nor the Taq1A2 restriction fragment length polymorphism located ~10 kb 3′ of *DRD2* were associated with overall abstinence in Caucasian smokers randomized to placebo or bupropion [71]. However, the Taq1A2 polymorphism was associated with bupropion-assisted quitting at 6-months follow-up [71]. In those with the *DRD2*-Taq1A2/A2 genotype, quit rates were higher on bupropion (versus placebo); by contrast, in Taq1A1 individuals, who are thought to have lower DRD2 receptor density [72], bupropion was not associated with greater cessation (versus placebo) [71]. Of note, the higher bupropion-assisted quit rates in those with the *DRD2*-Taq1A2/A2 genotype were restricted to those with *CYP2B6* rs3211371 TT or CT genotypes [71]. These findings highlight the potential importance of assessing multiple genes and gene–gene interactions, as opposed to single-gene analyses, to identify subgroups of smokers who are more likely to benefit from a certain treatment, discussed further below.

Optimizing Smoking Cessation through Multiple Genetic Predictors

Smoking cessation is influenced by a multitude of genetic and environmental factors (e.g., gender, socioeconomic status, and ethnicity) [73–75]. Treatment approaches that consider multiple predictors of cessation, versus single predictors, may show additional improvements in smoking cessation rates.

Chen and colleagues derived a four-level genetic risk score based on *CYP2A6* and *CHRNA5* in Caucasian smokers [32]. On NRT, but not bupropion, cessation outcomes differed according to risk score. In the highest-risk group (*CYP2A6* fast metabolism plus *CHRNA5* high-risk rs16969968-rs680244 diplotype), NRT had the largest treatment effect (versus placebo). Individuals with intermediate levels of risk showed an intermediate level of benefit from NRT, while individuals in the lowest-risk group (*CYP2A6* slow metabolism plus *CHRNA5* low-risk rs16969968-rs680244 diplotype) did not benefit from NRT [32].

An additive genetic efficacy score (AGES) has also been used to assess functional polymorphisms within the dopaminergic system and relapse risk in Caucasian smokers [76]. The AGES, calculated based on the number of alleles hypothesized to promote cessation on bupropion (versus placebo), comprised four variants selected based on their associations with smoking cessation [77]: *DRD4* exon-III VNTR, *SLC6A3* 3′ VNTR, and *DRD2* Taq1A (discussed previously), and *COMT* V158M (rs4680). In bupropion-treated smokers, AGES was not associated with time to first lapse [76]; however, bupropion mitigated relapse risk (versus placebo) in those with higher AGES scores, suggesting that these individuals should be treated with bupropion. The refinement of AGES and the development of other genetic scoring tools informed by biological pathways involved in smoking cessation and treatment pharmacology may show clinical utility in personalizing tobacco-dependence treatment. The addition of environmental influences on smoking cessation to such approaches may further improve cessation.

Concluding Remarks

In summary, a growing body of literature has demonstrated the importance of genes involved in the metabolism and central nervous system effects of both nicotine and smoking cessation medications in altering smoking cessation. Smokers with faster rates of nicotine metabolism, as determined by the NMR, have higher quit rates on varenicline versus the nicotine patch; by contrast, varenicline is not superior to the patch in slower metabolizers [22] (Figure 2). NMR genome-wide association studies have identified novel sources of genetic variation in CYP2A6 activity that may improve the optimization of treatment using *CYP2A6* genomics and phenotype (NMR) in the future [78]. Future studies should aim to determine: (i) the optimal NMR cut-point for distinguishing between faster and slower metabolizers (see Outstanding Questions); and (ii) whether the utility of NMR, as opposed to using a purely genetics-based approach, may be limited by transient environmental influences. Cessation studies in community-based smokers as well as cost–benefit analyses will enhance the implementation of NMR and other tools into clinical decision-making. Smokers with slower rates of bupropion metabolic activation to hydroxybupropion, as determined by *CYP2B6* genetics, may benefit from a higher dose of bupropion or alternative treatments [41]. Genetic variation in nAChRs and components of the dopaminergic system is also associated with smoking cessation; however, the findings from these studies have been less consistent, requiring further investigation to establish their potential for personalizing treatment. The development of treatment approaches that consider multiple genetic and environmental factors in tandem may provide additional improvements in smoking cessation rates and reductions in tobacco-related harm.

Outstanding Questions

Would the refinement of genetic risk scores, accounting for variation in multiple genes, improve the personalization of tobacco-dependence treatment?

How does pharmacogenetic variation in smoking cessation medication target genes influence treatment adverse effect profiles?

Will the pharmacogenetic findings from smoking cessation clinical trials replicate in community-based smokers, and in those with additional and/or psychiatric comorbidities?

Given that most pharmacogenetic investigations have been conducted in clinical trials in heavy smokers (10 + cigarettes/day), will these findings replicate in light smokers?

Given that many of the pharmacogenetic outcomes have been determined in smokers of Caucasian and African-American descent, will these pharmacogenetic findings replicate in smokers from other ethnic and cultural backgrounds and smoking phenotypes?

What is the optimal nicotine metabolite ratio cut-point to distinguish slower from faster nicotine metabolizers when attempting to optimize smoking cessation outcomes?

Could a more complete understanding of *CYP2A6* genomics lead to the development of a *CYP2A6* genetics algorithm to optimally treat nicotine dependence?

How feasible will it be to implement the nicotine metabolite ratio and/or *CYP2A6* genetics in clinical settings to tailor treatment choice and/or adjust treatment dose?

What mechanism(s) underpin the increased ability of CYP2A6 slow nicotine metabolizers to quit smoking?

Could *CYP2B6* genetic information be used to optimize bupropion dosing or the selection of an alternative smoking cessation treatment?

Acknowledgments

We acknowledge our sources of funding: Endowed Chair in Addictions (R.F.T.), National Institutes of Health (NIH) PGRN grant DA020830, Canadian Institutes of Health Research (CIHR) grants MOP86471 and TMH-109787, the Campbell Family Mental Health Research Institute of the Centre for Addiction and Mental Health (CAMH), the CAMH Foundation, the Canada Foundation for Innovation (#20289 and #16014), and the Ontario Ministry of Research and Innovation.

Disclaimer Statement

R.F.T. has consulted for Apotex. Funds were not received from Apotex for this work, and neither was the manuscript reviewed by individuals affiliated with Apotex.

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