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PHARMACOGENETICS OF NICOTINE ADDICTION: ROLE OF DOPAMINE

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Abstract

Brain dopamine (DA) plays a central role in addictive disorders including nicotine addiction. Several DA-related gene variants have been studied to identify predictors of pharmacotherapy response for smoking cessation. Genetic variants in *DRD2*, *DRD4*, *ANKK1*, *DAT1*, *COMT* and *DBH* genes show some promise in predicting response to smoking cessation pharmacotherapies. However, most of the clinical trials studying these variants had small sample sizes, used retrospective analysis, and enrolled smokers of self-identified Caucasians as study participants. In addition, future studies should also consider nicotine metabolism rate, sex or menstrual cycle phase, and epigenetic factors as potential confounding factors for treatment response of smokers. Future work addressing these limitations may uncover the potential impact of DA genetic variation on smoking cessation pharmacotherapies.

Keywords

Pharmacogenetics; Nicotine; Dopamine; Smoking Cessation; Genetic; Addiction

Introduction

Nicotine addiction continues to be the chief preventable cause of death in developed countries, causing an estimated 435,000 premature deaths in the U.S and 5 million deaths worldwide [1]. Quitting smoking is associated with immediate health benefits, irrespective of age or presence of smoking-related diseases [2]. Current first-line pharmacological treatments for smoking cessation (i.e., nicotine replacement therapy (NRT), bupropion, and varenicline) increase the chances of quitting smoking two to three fold. However, even when smokers utilize evidence-based cessation treatments, only 15%–25% of those who quit maintain tobacco abstinence for at least one year [3]. Thus, there is a great need to develop more effective treatments for nicotine addiction. Development of new treatments requires a better understanding of the individual factors contributing to different stages of nicotine

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addiction. Genetic factors are significant contributors to initiation and severity of nicotine addiction as well as response to treatment [4]. An estimated 50% of variance in nicotine addiction is explained by genetic factors although the specific genes contributing to this genetic component remain to be identified [5, 6].

This review article summarizes pharmacogenetics of smoking cessation with a focus on dopamine (DA), a neurotransmitter that plays a central role in addictive disorders including nicotine addiction [7]. First, we briefly review the neurobiology of nicotine's effects in the CNS, next we review genetic variations that directly or indirectly influence brain DA. Lastly, we review factors that may contribute to variation in pharmacogenetic studies for smoking cessation including sex and menstrual cycle, nicotine metabolism and epigenetic regulatory effects. The focus of this review is treatment studies. Several recent reviews are available, for a broader perspective of DA neurobiology [8] and pharmacogenetics of nicotine addiction [9].

Neurobiology of nicotine addiction

Nicotine, the main addictive chemical in tobacco smoke, is essential to continued and compulsive tobacco use [10]. Following a cigarette puff, nicotine enters cerebral circulation and binds to neuronal nicotinic acetylcholine receptors (nAChR). nAChR are ligand-gated ion channels which are permeable to sodium, potassium, and calcium ions. Most nAChR in the CNS are presynaptically located and modulate the release of acetylcholine (ACh), DA, serotonin, glutamate, gamma-aminobutyric acid (GABA), and norepinephrine [11]. nAChR can also be postsynaptically located, such as on the DA neurons in the ventral tegmental area (VTA). The two most commonly expressed nAChR in the brain are $\alpha 4\beta 2$ or $\alpha 7$ nAChR [11]. Stimulation of the $\alpha 4\beta 2$ nAChR located on the DA cell bodies in the VTA shifts these cells from tonic to phasic firing mode, which results in increased DA release in both the NAC and the prefrontal cortex. As with other drugs of abuse, this increased DA release in the NAC mediates the rewarding and pleasurable effects of nicotine and is presumed to be a critical mechanism for initiation and maintenance of nicotine addiction. Increased DA release in the prefrontal cortex is thought to be critical in mediating the cognitive-enhancing effects of nicotine.

Besides DA receptors, the NAC also contains GABA and glutamate synapses, which inhibit and stimulate nicotine-induced DA release, respectively. Prolonged exposure to nicotine is associated with desensitization and upregulation of nAChRs and the rates of these changes vary across nAChR subtypes (Figure 1). For example, the nAChR subtypes controlling GABA release (mainly non- $\alpha 7$ subtypes), desensitize faster than those controlling glutamate release (mainly the $\alpha 7$ subtype) [12]. This unequal desensitization may result in reduced GABA release, relative to glutamate, following prolonged nicotine exposure, such as in regular cigarette smokers. A relative deficiency of GABA over glutamate may lead to an enhanced DA release in the NAC and this may be a crucial mechanism that perpetuates nicotine addiction [12].

Another mechanism that contributes to nicotine reward occurs through nicotine-induced release of the endogenous opioid peptide, beta-endorphin [13]. Beta-endorphin stimulates

the mu opioid receptors (MOR) that are located on the GABA interneurons in the VTA, resulting in reduced GABA release. This reduction in GABA levels in the VTA, reduces the inhibition on the DA neurons, resulting in enhanced DA release in the NAC [14]

DA receptors

DA acts through five receptor subtypes (D1–D5) [15]. The DA receptors are also classified under two receptor families: D1-like (D1 and D5 receptors) and D2-like (D2, D3 and D4 receptors). These receptor families have opposing effects on signal transduction. Stimulation of D1-like receptors activates adenylate cyclase. In contrast, stimulation of D2-like receptors inhibits adenylate cyclase. The D2 receptor family also functions as an auto-receptor that reduces DA release. While the D1 and D2 receptors are the most commonly expressed DA receptors in the CNS, the D2 and D4 receptors have been the foci of pharmacogenetic studies for nicotine addiction.

DA D2 receptor gene (DRD2)

In *DRD2*, a single nucleotide insertion/deletion in the 5' promoter region (–141C Ins/Del) alters D2 expression [16]. As such, the deletion variant reduces D2 expression, which results in enhanced DA release, possibly due to decreased inhibitory auto-regulation [17]. Lerman et al. (2006) studied –141C Ins/Del in 368 smokers during an open-label 8 week trial comparing two forms of NRT (transdermal nicotine (TN) versus nicotine nasal spray (NS)) and observed that the –141C insertion allele improves quit rates independent of NRT type [18]. Dahl et al. (2006) extended the findings of Lerman et al. (2006) using the same open-label trial with 363 smokers, and observed an interactive effect between –141C allele and having two copies of a neuronal calcium sensor-1 protein single nucleotide polymorphism (SNP) A allele, rs1054879 [19]. Among 414 smokers participating in an 8 week double-blind placebo-controlled trial of bupropion, those with the –141C insertion had higher abstinence than those with the –141C deletion [18].

Lerman et al. (2006) also examined the influence of *DRD2* C957T (rs2283265) on smoking cessation outcomes. *DRD2* C957T is a synonymous SNP which modulates striatal D2 binding in vivo and mRNA stability in vitro, wherein the T allele is associated with higher *DRD2* availability [20, 21]. Smokers homozygous for the T allele of the C957T SNP who were on NRT experienced better smoking outcomes on NRT [18], while no genotype effect was observed in the group receiving bupropion responses.

The ankyrin repeat and kinase domain-containing (*ANKK1*) gene contains a functional SNP previously known as *DRD2* TaqI (rs1800497). Although this SNP is located 10 kb downstream from *DRD2*, individuals who carry the *ANKK1* A1 allele have a 40% reduction of D2 receptor density compared to those who are homozygous for the A2 allele [22]. Several studies suggest that *ANKK1* A1 and A2 alleles may influence smoking cessation outcomes. Johnstone et al. (2004) reported that among 755 heavy smokers who participated in a 12 week double-blind, randomized, controlled trial of NRT, nicotine patch was more effective for smokers who carried at least one *ANKK1* A1 allele at week 1. At week 12, smokers who carried both the *ANKK1* A1 allele and dopamine beta hydroxylase (DBH) 1368A (rs77905) allele had both the highest cessation rates on NRT and the lowest quit rates

on placebo [23]. In a reanalysis of a sample which overlapped with Johnstone et al's., (2004) sample, Yudkin et al. (2004) observed that woman with at least one copy of the *ANKK1* A1 allele had better response to NRT compared with women without the A1 allele [24].

Many smoking cessation pharmacogenetics studies have investigated interactions between bupropion and *ANKK1*. David et al. (2003) studied whether *ANKK1* moderated the effects of bupropion in a randomized, double-blind, placebo-controlled smoking cessation study with 29 heavy smokers. The greatest reductions in craving, anxiety and irritability were observed in smokers carrying the A2/A2 genotype who were on bupropion [25]. This type of pharmacogenetic effect was also observed in a sample of 218 smokers during a randomized, double-blinded, placebo-controlled trial of bupropion for smoking cessation[26]. Smokers carrying the A2/A2 genotype experienced larger reductions in craving and greater likelihood of abstinence on bupropion. Additionally, those who carried A2/A2 allele plus the T allele from a functional CYP2B6 SNP (rs3211371) achieved more abstinence [26]. The same research group also pooled their data [26] with pharmacogenetic work from Lerman et al., (2003) for a combined sample of 722 smokers to determine whether the *ANKK1* SNP rs1800497 moderated treatment response to bupropion for smoking cessation [27]. The results from the combined sample indicated that those who carried the A2/A2 genotype and were given bupropion were more likely to be abstinent at the end of treatment compared with placebo [28]. In an 8-week open-label of treatment with bupropion sustained-release (SR) with 451 smokers in the pharmacogenetic component of the study, Swan et al (2005) found that smokers who carried the A1 allele were more likely to report discontinuing bupropion because of side effects [29]. One clinical trial evaluating the effects of an antidepressant medication (venlafaxine or placebo) plus standard care in 134 smokers observed that those carrying the *ANKK1* A2/A2 genotype quit significantly more often and experienced a significant reduction in reduced negative mood symptoms compared with those with the A1 allele, regardless of medication condition [30].

Collectively these studies report that *DRD2* may be an important moderator of smoking cessation, especially in smokers who have the A2/A2 genotype on bupropion, while those who carry the A1 allele seem to report more side effects from the medication [25–27, 29]. *DRD2* and the proximal *ANKK1* remain potential pharmacogenetic targets. The studies conducted seem to implicate this region as moderating smoking cessation pharmacotherapies however these reports contain issues that need to be addressed in future work. For example, the samples collected were primarily Caucasian and usually not confirmed through measurement of genetic ancestry. Additionally, more stringent correction for multiple testing should be employed. At least three reports on the pharmacogenetics of smoking cessation and *DRD2* utilize nearly the exact same sample and it is unclear whether stringent statistical methods were employed to guard against Type I errors.

DA D4 receptor gene (DRD4)

The structure and pharmacology of the D4 receptor is similar to the D2 receptor. The *DRD4* gene is predominantly expressed in the prefrontal cortex and has been widely examined in relation to psychiatric disorders, including nicotine addiction. The *DRD4* gene contains two polymorphisms that have been studied in relation to nicotine pharmacogenetics. The first

polymorphism of interest is located in exon 3 where a 48 base pair (bp) variable number tandem repeat (VNTR) encodes the third intracellular loop of the receptor [31, 32]. The 7-bp repeat variant displays decreased sensitivity to DA and differences in DA binding potential compared to other common variants (2-bp or 4-bp repeats) [33].

In a randomized placebo-controlled trial of NRT with 720 smokers, David et al (2008) found that those who had at least one copy of 7-bp repeats were less successful in quitting smoking at 12 weeks than those with no copies of 7-bp repeats, irrespective of treatment assignment [34]. However, this genetic effect on outcomes dissipated at 26 weeks. Leventhal et al., (2012) studied 331 smokers in a double-blind placebo-controlled 12 week randomized trial of treatment with bupropion. Smokers who were treated with bupropion and had at least one copy of 7-bp repeats, compared to those without any 7-bp repeat copies, were more likely to quit smoking at 2, 6 and 12 months after treatment ended [35], in contrast to the findings of David et al (2008). Bergen et al., (2013) utilized data from the Lerman et al., (2003) double-blind pharmacogenetic efficacy trial randomizing 416 smokers to active or placebo bupropion [36]. While they did not observe statistically significant effects of genotype or genotype by treatment interactions, they conceded that the lack of pharmacogenetic effects in their study may have been due to insufficient sample size.

A second relevant *DRD4* genetic variation is a C-to-T transition, located 521-bp upstream from the transcription start site, wherein the T allele reduces transcriptional efficacy by 40% compared with the C allele. One study investigating this SNP in 720 smokers in a double-blind, randomized, placebo-controlled trial of NRT observed no pharmacogenetic effects [34, 37].

The D4 receptor plays a critical role in the neurobiology of DA transmission and, therefore, the rationale of studying *DRD4* gene variation as a moderator of smoking cessation pharmacotherapies is sound. However the results from existing clinical trials generally do not support roles for these most well-characterized *DRD4* gene polymorphisms in response to smoking cessation pharmacotherapy.

Dopamine transporter gene (*DAT1*, or *SLC6A3*)

The DA transporter (DAT) terminates DA neurotransmission in subcortical regions through re-uptake of synaptic DA to the presynaptic neurons. DAT is the site of action for stimulant drugs like cocaine, amphetamine, and methylphenidate. *DAT1*, composed of 15 exons on chromosome 5p15.3, has few coding region variants. Therefore, most *DAT1* genetic studies have focused on non-coding SNPs and variable number tandem repeats (VNTR) [38, 39]. The 3' untranslated region contains a VNTR varying between 3 and 12-bp repeats with the most frequently observed alleles containing 9 and 10-bp repeats. The 10-bp repeat allele has been associated with an abnormally active DAT, eliciting increased re-uptake of DA and DA degradation leading to reduced DA transmission [40].

In a placebo-controlled trial, this VNTR variation did not moderate bupropion's smoking cessation effects in 418 smokers (Lerman 2003). However, the same study reported that smokers who carried both the 9 repeat allele and the *ANKK1* A2 allele had longer successful abstinence before relapsing to smoking, regardless of treatment condition [41].

O’Gara et al. (2007) conducted an open-label trial of bupropion versus NRT treatment, that both included behavioral support, in 583 smokers and observed that the 9 repeat VNTR was associated with higher rates of quitting smoking with either treatment [42]. In contrast, in an open-label clinical trial, bupropion paired with smoking cessation counseling in 416 smokers [43], those who carried the 9 repeat allele (compared to those with other alleles) had poorer smoking cessation outcomes at the 12 month study follow up. A separate randomized placebo-clinical trial on 291 smokers found no effect of DAT1 VNTR on bupropion’s efficacy for smoking cessation [26].

The results of the *DAT* smoking cessation pharmacogenetic studies are equivocal. The samples sizes of these studies are modest but the studies are more numerous compared with DA smoking cessation pharmacogenetic studies looking at other genetic regions (*DRD2*, *COMT*, etc.). *DAT1* remains a good candidate gene for moderating the therapeutic effects of smoking cessation and should be of particular interest in relation to bupropion, given bupropion’s selectivity for binding to DAT. Similar to other studies on DA pharmacogenetics for nicotine addiction, the majority of the available studies enrolled only Caucasians, so it is not currently known whether these effects generalize into ethnicities with significantly different genetic background than Caucasians.

DA synthesis and inactivation: tyrosine hydroxylase (TH), dopamine-beta-hydroxylase, monoamine oxidase (MAO), catechol-0-methyl-transferase (COMT) genes

Tyrosine hydroxylase converts tyrosine to dihydroxyphenylalanine, the rate-limiting step for DA biosynthesis. Monoamine oxidase (MAO) and catechol-0-methyl-transferase (COMT) convert DA into homovanillic acid (HVA). Both MAO and COMT are found in monoamine neurons and glial cells. Dopamine-beta-hydroxylase (DBH) converts DA to norepinephrine in the synaptic vesicles of the noradrenergic neurons [44], thereby causing synaptic DA levels to increase, relative to norepinephrine. Among these genes important for DA synthesis and inactivation, only *COMT* and *DBH* have been examined in pharmacogenetic studies for nicotine addiction.

COMT

COMT contains a well-studied mis-sense SNP (Val¹⁵⁸Met) that results in the presence of methionine (Met) or valine (Val) at codon 158 in the membrane-bound COMT. The Val allele is three to four times more active than the Met-coded allele, resulting in reduced DA levels in the synapse. In a clinical trial of 290 Caucasian and Black female smokers, those with the Met/Met genotype were more likely have prolonged abstinence in response to NRT treatment compared with women who carried the Val/Val genotype [45]. These results were replicated in a randomized placebo-controlled NRT trial, where (N=749) Caucasian smokers with the Met/Met allele were more likely to be abstinent than smokers carrying at least one Val allele, regardless of treatment condition [46]. In contrast, a double-blind, placebo-controlled, 8-week trial of NRT with 250 Chinese smokers, those with the Val/Val genotype, compared with those who carried at least one Met allele, had better abstinence rates with NRT [47]. Recently, we examined if COMT moderates the behavioral responses

to intravenously administered nicotine in 124 Black and Caucasian smokers. Smokers who carried the Val/Val genotype experienced more negative subjective effects from nicotine and had higher withdrawal severity following overnight abstinence from smoking [48]. These findings provide a potential mechanism by which Val/Val genotype may be associated with greater difficulty quitting smoking.

COMT genetics has been associated with bupropion efficacy for smoking cessation. Berrittini et al. (2007) studied the Val/Met polymorphism and two additional SNPs (rs737865 [located in the first intron of the MB-COMT transcript] and rs165599 [near the 3' untranslated region]) that have shown evidence for differential allelic expression during a double-blind, placebo-controlled, 10-week trial of bupropion in 430 Black and Caucasian smokers. Quit rates were better for smokers who carried the rs165599 GG genotype on placebo, but were better for A genotype carriers on bupropion [49]. In a sample of 250 Korean smokers who participated in an open-label bupropion trial, Han et al. (2008) found the Val/Val genotype was more commonly associated with smoking abstinence. The authors cautioned that the pharmacogenetic effect did not withstand correction for multiple testing [50].

DBH

DBH contains multiple well-characterized polymorphisms that explain significant proportions of *DBH* activity. DBH1368 is in tight linkage disequilibrium to a G-to-A substitution associated with higher *DBH* levels [51]. Johnstone et al. (2004) tested whether DBH1368 genetic variants moderate responses to transdermal NRT. The results of this study indicated that smokers who carried the 1368 *DBH* A allele, combined with ANKK1 Taq 1A, experienced better NRT efficacy after 12 weeks of treatment [23].

Genes that control DA synthesis and inactivation remain particularly promising as pharmacogenetic moderators of smoking cessation. Studying the pharmacogenetics of *COMT* in both female and male samples [45] is important given that *COMT* exhibits sexual dimorphisms regulated by estrogens [52]. Future work will require controlling for sex and estradiol levels. Additionally, *COMT* effects are differentially regulated by ethnicity and therefore collecting representative samples from distinct genetic ancestries may be needed. To our knowledge, there have been no smoking cessation pharmacogenetics studies that have investigated MAOA, MAOB or TH genetics and there is scant data for DBH.

Future perspectives

Future work of innovating personalized medicine approaches for smoking cessation will likely require more nuanced research than simply studying a single gene variation and pharmacotherapy response during a clinical trial. The most recent evidence suggest that race/ethnicity, sex, menstrual cycle phase, nicotine metabolism rate and epigenetic regulation may all contribute to individual differences in nicotine addiction and may need to be considered in nicotine pharmacogenetic studies.

Race and ethnicity

The majority of smoking cessation pharmacogenetic studies have recruited predominantly Caucasian samples and despite race and ethnicity appearing to be related to multiple aspects of nicotine addiction. For example, in the United States, Blacks smoke fewer cigarettes, compared to Caucasians, but have higher levels of nicotine dependence. Compared to Caucasians, Blacks are more likely to attempt to, but less likely to succeed in, quitting smoking [53, 54]. Asians from China have smoking prevalence rates of 28.1%, yet along with Latinos, have the lowest rates of lung cancer [55]. Genetic variation could in part help explain why individuals from different ethnicities have significantly different rates of smoking and health-related outcomes.

Research as early as the 1950s reported racial/ethnic differences in drug responses. One of the best examples comes from research in cardiovascular disease. In the early 1980's, clinical observations reported that response to antihypertensive drugs differed by race such that self-identified Caucasians experienced higher response to beta-adrenergic receptor antagonists (i.e., beta-blockers) compared with self-identified Blacks. Subsequently, researchers have observed functional genetic variations in the genes encoding the beta-adrenergic receptors, which are the targets of this class of drugs [56]. The same variants often show marked differences in frequency among world populations, such that individuals of primarily Caucasian ancestry are more likely to carry the alleles more responsive to beta-adrenergic receptor antagonists at these sites in comparison with individuals of Black ancestry [57].

Importantly, the treatment of race/ethnicity as a proxy for ancestry is not a valid approach [58]. Most of nicotine pharmacogenetics studies that have been published typically only include individuals who self identify themselves as Caucasian. The studies that have included individuals from multiple backgrounds commonly neglected to properly assess genetic ancestry. Given the prior successes of harmonizing ethnic ancestry with pharmacotherapy responses (e.g., beta-blockers and warfarin), future smoking cessation pharmacogenetic trials should include genetic ancestry.

Sex and menstrual cycle

Women respond less favorably to smoking cessation treatments [59], even though they maintain their nicotine addiction with lower levels of nicotine intake than men [60]. Data from multiple smoking cessation clinical trials have suggested that sex may critically modulate pharmacogenetic effects. For example, women, but not men, with at least one A1 allele experienced greater benefits from transdermal NRT, compared to women homozygous for the A2 allele [24]. In another study, Swan et al. (2005) evaluated the role of ANKK1 in an open-label, randomized effectiveness trial comparing low and high doses of bupropion. Compared to women homozygous for the A2, women with at least one A1 allele were less likely to quit smoking with bupropion treatment [29,34 5]. However, this genotype effect on treatment response was not observed in men. Further, in a smoking cessation trial of transdermal nicotine and nicotine nasal spray, presence of the Met allele of COMT facilitated smoking cessation in women [45]. Additionally, females with the Val/Val genotype experienced greater difficulty in concentrating and reported more irritability than

men who were in either Val/Val or Met genotype groups [48]. In contrast to these findings, other studies did not report sex differences in DA pharmacogenetic studies of smoking cessation have not reported sex differences [35, 46, 61].

In a recent study, we observed that female smokers (n=49), relative to males (n=111) had greater physiological responses to, yet diminished subjective drug effects from intravenous nicotine administration. Among women, smokers who were in the luteal phase (progesterone dominant), showed attenuated subjective drug effects, less withdrawal, less change in cravings following the session, and better cognitive performance relative to women who were in the follicular phase (estradiol dominant) [48]. Our findings are consistent with the activation and inhibition of the DA reward pathway by estradiol and progesterone, respectively [62]. Our findings also suggest that cyclic changes in estradiol and progesterone levels that occur across the menstrual cycle may contribute to the observed sex differences in nicotine's effects. These findings offer several candidate mechanisms through which the luteal phase, wherein progesterone is dominant relative to estradiol, may be protective against vulnerability to smoking. Other studies have also reported that the phase of the menstrual cycle may influence smoking cessation outcomes [63]. Most of the smoking cessation pharmacogenetic studies published to date have not included sex hormones as covariates in their analyses. Introducing these biological measures in subsequent trials could further clarify the role of sex differences in pharmacogenetics smoking cessation studies.

Nicotine metabolism

Nicotine is inactivated in the liver to its main metabolite, cotinine, primarily by the CYP2A6 microsomal enzyme [64, 65]. The same enzyme oxidizes cotinine to 3'-hydroxycotinine (3HC). The gene encoding the CYP2A6 enzyme is highly polymorphic, and multiple functional allelic variations and SNPs have been described [66]. Many studies have demonstrated that the ratio of plasma 3HC/cotinine correlates with nicotine clearance [67, 68]. Because the nicotine metabolite ratio correlates with nicotine clearance and CYP2A6 genotype [66, 69], it is considered to be a marker of CYP2A6 activity [68].

The nicotine metabolite ratio may also be predictive of smoking cessation outcomes. Smokers in the lowest ratio quartile (slower metabolizers), compared to those in higher quartiles, show better smoking cessation outcomes with transdermal NRT [70]. In another clinical trial that tested the efficacy of bupropion for smoking cessation, smokers in the fourth quartile (faster metabolizers) had poor smoking cessation rates if they were assigned the placebo treatment relative to slower metabolizers, however bupropion lowered the relapse liability among high metabolite ratio smokers [71]. Overall, these findings are consistent with the idea that smokers who rapidly metabolize nicotine might be more dependent and have greater difficulty with smoking cessation [72]. The underlying mechanisms that associate nicotine metabolite ratio with treatment outcomes have not been fully elucidated. In a recent study, we have shown that, higher nicotine metabolite ratio was associated with a greater craving for cigarettes and a greater reward from intravenous nicotine following overnight abstinence in smokers [73]. These results suggest that enhanced nicotine reward and cigarette craving may contribute to the poor treatment

response in smokers with high nicotine metabolite ratio. Studies are underway to test the utility of the nicotine metabolite ratio for smoking cessation.

Epigenetic regulatory effects

The effects of DA gene variants might be modulated by epigenetic mechanisms. Understand these important effects might help to clarify the role of dopamine in the pharmacogenetics of nicotine addiction and lead to better treatments. Epigenetic effects involve changes to chromatin structure caused by direct covalent modifications of DNA or factors that interact with DNA, such as histones. These changes to DNA structure might influence the spatial or temporal pattern of gene expression. Exposure to addictive drugs [74, 75] and early life stress [76] have effects on chromatin structure, including some effects that are stable with long-term consequences on brain function and behavior. Exposure to nicotine, specifically, may influence vulnerability to development drug dependence through epigenetic mechanisms. In rodent studies nicotine acts as a chromatin modifier by reducing the expression of mRNA from multiple histone methyltransferase genes and the level of dimethylated histone H3 lysine 9 (H3K9me2) [77]. Gozen et al. showed that a 15-day nicotine exposure induced DRD1 mRNA expression in the prefrontal cortex (PFC). The increase in DRD1 mRNA expression occurred with a concomitant increase in the fraction of acetylated histone H4 at the DRD1 gene promoter [78]. In a rodent model, Amir et al. investigated the effects of nicotine pretreatment on response to cocaine. They found that nicotine increased the level of acetylated histone H3 and H4. The increased levels of histone acetylation correlated with increased sensitivity to the addictive properties of cocaine [79]. These findings may have important clinical implications given the frequent comorbidity of nicotine with substance dependence.

Conclusions

Many studies have examined the contribution of DA-related genes in smoking cessation outcomes. The genes that have been evaluated include *D2*, *D4*, *DBH*, *COMT* and *DBH*. Many initial positive findings could not be replicated by other researchers. This lack of replication could be due to multiple testing [80], differences in outcome measures across studies and small sample sizes, ranging from 29 to 755. Future studies should also take into account several confounding variables such as sex, menstrual cycle phase, ancestry, nicotine metabolite ratio and epigenetic regulatory effects.

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Executive summary

Clinical relevance of pharmacogenetics of nicotine addiction

- Despite dire health consequences of nicotine addiction and immediate health benefits of quitting smoking, the majority of cigarette smokers who attempt to quit are unsuccessful.
- Pharmacogenetic approaches may help to enhance the efficacy of currently available smoking cessation pharmacotherapies.

DA plays a central role in nicotine addiction

- When nicotine binds to neuronal nAChR, DA is released, along with other neurotransmitters.
- Nicotine-induced DA release, in regions including the nucleus accumbens, is thought to be a critical mechanism for initiation and maintenance of nicotine addiction.
- Given the central role of DA in nicotine addiction, variation of genes related to DA function may serve as potential targets for pharmacogenetic approaches for nicotine addiction.

Key DA-related polymorphisms that may moderate nicotine addiction pharmacotherapies

- *DRD2* C957T (rs2283265), a synonymous SNP, modulates striatal dopamine D2 receptor binding and mRNA stability.
- *DRD2* (-141C Ins/Del) a single nucleotide insertion/deletion in the 5' gene promoter region alters D2 expression. The deletion variant reduces D2 expression, leading to greater DA release.
- *ANKK1* (rs1800497) A1 allele reduces D2 receptor density 40% compared to presence of the homozygous A2 allele.
- *COMT* (rs4680) Val158Met polymorphism influences dopamine inactivation, with the valine (Val) variant catabolizing dopamine at up to four times the rate of its methionine (Met) variant.

Evidence indicating clinical meaningful nicotine addiction pharmacogenetic effects

- Smokers who carry the *DRD2* A2 allele had better smoking cessation outcomes and fewer side effects while on bupropion, relative to those who carry the *DRD2* A1 allele.
- *COMT* Val158Met variation may moderate response to NRT. However, two nicotine replacement trials found better outcomes with smokers who carried the Met/Met genotype and two found better outcomes for Val/Val.
- Several other DA-related genetic polymorphisms (e.g., *DRD4* VNTR, *DAT1* VNTR) have been investigated in the context of clinical trials for smoking cessation, with equivocal findings.

Future directions

- Previous clinical trials examining the role of DA-related genes as predictors of pharmacotherapy response had several limitations including small sample sizes, retrospective analysis, and composed of mainly self-identified Caucasians as study participants.
- Evidence suggests that nicotine metabolism rate, sex or menstrual cycle phase, and epigenetic factors are potential confounding factors for treatment response of smokers. These factors should be considered in future pharmacogenetic studies for smoking cessation.

Conclusion

- Gene variants in the DA system remain relevant in improving the efficacy of pharmacotherapies for smoking cessation.
- Future work addressing the limitations of previous studies may uncover the potential impact of DA genetic variation on smoking cessation pharmacotherapies.

Figure 1.

A simplified illustration of the dopamine (DA) neuron in the ventral tegmental area (VTA) and the hypothesized effects of nicotinic acetylcholine receptors (nAChR) on the regulation of dopamine (DA), glutamate and GABA release in the VTA and nucleus accumbens.

Abbreviations: DAT: Dopamine transporter; DOPA: Dihydroxyphenylalanine; DOPAC: 3,4-Dihydroxyphenylacetic acid; HVA: Homovanillic acid; MAO: Monoamine oxidase; MOR: Mu opioid receptor; TH: Tyrosine hydroxylase.

Table 1
Pharmacogenetic and Genetic Effects of Dopamine-related Genes on Smoking-cessation Outcomes

DA System	Gene	Allelic variant	Genetic	Medication	Pharmacogenetic	Summary
D2 receptor	DRD2	-141C	+	NRT	-	Genetic effects were found for -141C and C957T in the context of NRT and bupropion trials. Insertion (vs. deletion) variant of -141C had better smoking outcomes [21], but in a second study this effect was only observed when -141C insertion was paired with rs1054879A [22]. The TT (vs. CT or CC) variant of C957T had better smoking outcomes in individuals receiving NRT but not amongst those receiving bupropion [21]. Since no formal medication by genotype interactions were observed, direct evidence for pharmacogenetic effects of these DRD2-related allelic variants for NRT or bupropion is lacking.
				Bupropion	-	
		C957T	+	NRT	-	
				Bupropion	-	
D4 receptor	ANKK1	rs1800497	+	NRT	+	Differential pharmacogenetic effects of rs1800497 have been observed for NRT and bupropion. NRT smoking outcomes were better for smokers with A1 alleles in mixed-gender or female-only analyses of an overlapping sample [26, 27]. In contrast, bupropion smoking outcomes were better for smokers with A2/A2 alleles [28, 29, 30]. A study of venlafaxine found no pharmacogenetic effects; yet found genetic effects favoring smoking and mood outcomes in A2/A2 smokers [33].
				Bupropion	+	
				Venlafaxine		
Dopamine transporter (DAT)	DRD4	VNTR	+	NRT	-	Differential genetic effects of DRD4 VNTR were observed for NRT and bupropion. In an NRT trial [37], smokers with <7bp had better smoking outcomes. However, in the context of bupropion trials, smokers with 7bp had better [37] or a non-significant tendency towards better [38] outcomes. No evidence of genetic or pharmacogenetic effects of DRD4 C-to-T variant was observed on smoking outcomes with NRT [37, 40]. No formal medication by genotype interactions were reported, so evidence of DRD4-related pharmacogenetic effects is limited.
				Bupropion	-	
		C-to-T	-	NRT	-	
DA inactivation	COMT	Val ¹⁵⁸ Met	+	NRT	+	Findings are mixed regarding which variants are beneficial for smoking outcomes. Two studies including a mixed-race female only sample [48] or Caucasian-only mixed-sex sample [49] found better outcomes for Met/Met carriers on NRT. However, in a Chinese-only sample, Val/Val was associated with better NRT outcomes [50]. Similarly, a trend towards better outcomes for Val/Val carriers on bupropion was reported in a Korean-only sample [52]. For rs165599, A carriers had better outcomes on bupropion [50]. Genetic, but not pharmacogenetic, effects were found with Val/Val experiencing worse withdrawal symptoms [62]. The bulk of findings support pharmacogenetic effects of COMT on NRT and bupropion. The variable findings may be partially attributable to race/ethnicity and sex differences across the samples.
				Bupropion	+	
				Bupropion	+	
		rs737865	-	Bupropion	-	
	DBH	DBH1368	-	NRT	+	The only study assessing DBH1368 found better outcomes on NRT for A (versus G) carriers, but this effect was conditional on the presence of ANKK1 Taq1A allele [26].

TH=tyrosine hydroxylase; VNTR=variable number tandem repeats; DA=dopamine; SNP=single nucleotide polymorphism; bp= base pairs; NRT= nicotine replacement therapy; pharmacogenetic effects represent gene variant by medication interactions on better smoking outcomes; genetic effects represent gene variant main effects on better smoking outcomes. C957T is also known as rs2283265. rs1800497 is also known as Taq1. DAT1 is also known as SLC6A3. '+', '-' indicates evidence in support of an effect; '+', '-' indicates that despite studies testing this relationship, no effect was indicated.