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Genetics of nicotine dependence and pharmacotherapy

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ABSTRACT

Nicotine dependence is substantially heritable. Several regions across the genome have been implicated in containing genes that confer liability to nicotine dependence and variation in individual genes has been associated with nicotine dependence. Smoking cessation measures are also heritable, and measured genetic variation is associated with nicotine dependence treatment efficacy. Despite significant strides in the understanding of the relative contribution of genetic and environmental factors to nicotine dependence and treatment, emergent challenges necessitate interdisciplinary coordinated effort for effective problem solving. These challenges include refinement of the nicotine dependence phenotype, better understanding of the dynamic interplay between genes and environment in nicotine dependence etiology, application and development of molecular and statistical methodology that can adequately address vast amounts of data, and continuous translational cross-talk.

1. Preface

Critics suggest that genetic research on cigarette smoking behavior will not add to the gains in reducing the prevalence and incidence of smoking being made by public health approaches [1,2]. It is widely accepted that prevention and policy efforts such as no smoking policies and high tax rates on tobacco products are vital in deterring early stages of smoking, such as starting to smoke, and are likely to prompt less dependent smokers to quit. At the same time, one might hypothesize that as the environment surrounding smoking behavior becomes more restrictive leading to reduced smoking behavior, the genetic variation explaining smoking behavior will change accordingly [3]. This may, therefore, be the opportune time to examine change in genetic versus environmental influences across cohorts and stages of smoking, in addition to also examine important gene by environment interactions.

Genetic research on smoking behavior shows that individual differences in smoking can be attributed to hundreds (if not thousands) genetic and environmental factors. With few exceptions, the primary goal of genetic research on smoking behavior is not "genetic testing" or immediate clinical application as some critics have implied [1]. Instead, work in this area can be characterized by at least two long-term goals. The first goal is to better understand the etiology of nicotine dependence, which is classified as a medical disorder by the Diagnostic and Statistical Manual of Mental Disorders [4]. The second goal is to identify functional genetic variants which could, in conjunction with other treatment factors, inform future smoking cessation treatment approaches. Twin studies, for example, provide one avenue through which genetic and environmental factors on smoking are considered simultaneously. Theoretically, twin studies offer the ability to test for dynamic shifts in genetic and environmental influences across cohorts and across the development of nicotine dependence.

In this review, we summarize some of the most recent evidence for genetic influences on smoking behavior, with specific emphasis on nicotine dependence and smoking

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cessation-related phenotypes. We describe measures of nicotine dependence that are commonly used for phenotypic characterization and present the issue of how variation in the phenotype can influence genetic results. Next, we provide an overview of findings on the genetic and environmental influences on the etiology of nicotine dependence including results from twin, family and adoption studies, and linkage and association studies. We then present findings on gene variation associated with smoking cessation treatment response. Finally, we highlight issues to consider within the context of genetic research on smoking with emphasis on the importance of communication between public health experts, epidemiologists, neurobiologists, molecular and statistical geneticists, and clinical researchers to synthesize, integrate, and translate generated data. Throughout we also discuss the challenges, limitations, and future direction of the field.

2. Introduction

Cigarette smoking, the most common form of tobacco use [5], is the single most preventable cause of lung cancer [6] and is a significant source of morbidity and mortality worldwide [7]. Despite disastrous health consequences from smoking, 21.6% of American adults continue to smoke cigarettes [8]. Close to half of current US smokers (42.5%) report that they quit smoking for at least one day in the previous year [9]. However, clinical trials show that long term abstinence rates of 6 months or longer are low, averaging 10% [10], suggesting high relapse rates. Nicotine dependence, among other factors, contributes to maintaining smoking behavior; more highly dependent smokers have greater difficulty quitting smoking cigarettes [11,12]. Average quit rates increase with pharmacological treatment such as nicotine replacement therapy (13.7–24.0%) [13], the antidepressant bupropion (14–19%) [10], and the selective $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist [14] varenicline (20–36.7%) [15–17]. However, long-term abstinence following treatment is still rare.

Low rates of successful smoking cessation may be in part due to constitutional factors, such as genetic predisposition to nicotine dependence. The evidence for a significant role of genetic factors on nicotine dependence is substantial. Nicotine dependence and associated characteristics are highly heritable [18–28]. Linkage studies have identified candidate genomic regions that may contain genes associated with nicotine dependence [29–32] and gene association studies have identified candidate genes associated with nicotine dependence [33–35]. Finally, measured variation in specific genes is associated with differences in response to smoking cessation treatment [36–39], suggesting that pharmacogenetic approaches hold promise for personalized treatment by increasing success rates in nicotine dependence treatment [40,41].

3. Defining nicotine dependence

The importance of defining the phenotype is particularly salient when it comes to identifying the genetic mechanisms responsible for explaining phenotypic variation [42]. Different definitions of nicotine dependence will include smokers with varying smoking histories and, therefore, at varying genetic risk for nicotine dependence, which will alter sample variance and will impact estimates of heritability, genetic association, and linkage results [43,44]. Defining nicotine dependence has been quite challenging especially in regards to defining non-smokers. For example, should an individual who has experimented with cigarettes but has not continued using cigarettes beyond experimentation be considered a non-smoker? Is someone who has experimented with cigarettes and, perhaps, smoked 10 cigarettes in their life but never progressed to a regular smoking pattern a non-smoker? Should these categories of 'non-smokers' be considered non-dependent or non-informative (and therefore missing data) for nicotine dependence categorization? Recent data show that measurable dependence symptoms in adolescents emerge as soon as two days following the first puffs of a cigarette and as infrequently as smoking seven cigarettes per month [45]. These results replicate previous findings by the same group in a different cohort of adolescents [46]. In another study, adolescents reported experiencing ''mental addiction'' within three months of the first cigarette inhalation and experiencing ''cravings'' and ''physical addiction'' very soon after smoking a whole cigarette and before onset of just smoking once or twice per month [47]. Results from these studies suggest that symptoms of nicotine dependence can be observed at very low and infrequent levels of smoking. Therefore, it seems that (1) it may be inappropriate to define individuals who have experimented with cigarettes but have not progressed to a regular smoking pattern as non-smokers, since these individuals may have experienced symptoms of dependence; and (2) common survey practice of skipping individuals who have not met a pre-determined threshold of smoking history (e.g., regular smoking) from assessment of nicotine dependence may miss important information in overall variability in dependence risk. On the other hand, if an individual has never tried cigarettes (i.e., they have never been exposed to nicotine) then their degree of genetic risk is unknown, and setting their responses to missing may be appropriate in most cases.

3.1. *Instruments used for assessment of nicotine dependence*

One of the earliest instruments aimed at assessing nicotine dependence was the Fagerström Tolerance Questionnaire (FTQ) [48], an 8-item paper–pencil survey that was developed specifically to assess physical dependence to nicotine in smoking cessation clinic patients. Two of the eight FTQ items assessing the time to first cigarette of the day after waking up and the number of cigarettes smoked per day were shown to explain a significant proportion (17–20%) of the total variance of the FTQ [49]. The original scoring of these two items was expanded into categories that were superior in predicting biochemical and behavioral indices of smoking and these measures were combined as the Heaviness of Smoking Index (HSI) [50]. The HSI categorization was used in a revision of the FTQ to a more psychometrically sound 6-item version, the Fagerström Test for Nicotine Dependence (FTND) [51], which has become a commonly used nicotine dependence assessment tool in epidemiological surveys, clinical trials, and

laboratory studies. The FTND has been found to be unidimensional in some studies [51,52], but others have reported a two-factor structure comprising of morning smoking and daytime smoking factors [53–59].

A second commonly employed instrument for nicotine dependence assessment is diagnostic nicotine dependence as defined by the Diagnostic and Statistical Manual for Mental Disorders, 3rd edition Revised and 4th edition (DSM-III-R and DSM-IV) [4,60]. With the exception of nicotine-specific withdrawal symptoms described in the DSM-IV, dependence criteria were developed so that they can be applicable to a wide range of substances [61], thus the advantage of the FTQ over the diagnostic criteria is that it was developed specifically to assess physical dependence to nicotine. Diagnostic nicotine dependence appears to capture a dependence factor associated with tolerance and general cigarette use and a second factor associated with withdrawal and difficulty quitting [21,62,63]. The correlations between the FTQ/FTND and diagnostic nicotine dependence are low to moderate [64–66] suggesting that these instruments capture different dimensions of nicotine dependence.

The general substance use items that comprise DSM-IV nicotine dependence include: (1) tolerance, (2) withdrawal, (3) using more of the substance than intended, (4) persistent desire to quit or unsuccessful efforts to cut down, (5) great deal of time spent obtaining the substance (e.g. chain smoking), (6) important activities given up or reduced because of substance use, and (7) continued substance use despite persistent or recurrent physical or psychological problem caused or exacerbated by its use. Thus, for DSM-IV defined nicotine dependence, the individual has begun to view their behavior as a ''problem'' affecting their life in some way. In contrast, FTND items are somewhat more objective: (1) time to first cigarette after waking, (2) difficulty refraining from smoking in place where it is forbidden, (3) would most hate to give up first cigarette in the morning versus all others, (4) number of cigarettes smoked per day, (5) smoking more frequently during the first hours after waking than during the rest of the day, and (6) smoking when so ill that you are in bed most of the day. It remains to be seen whether the development of the DSM-V will branch out to include substance-specific criteria, which would have important implications for phenotypic refinement.

Recent instrument development has focused on capturing the multidimensionality of nicotine dependence symptoms that are more specific to problems associated with tobacco use as opposed to broad substance dependence criteria. The Nicotine Dependence Syndrome Scale (NDSS) was developed as a DSM-IV-based multidimensional instrument specific to assessment of nicotine dependence [67]. The NDSS comprises of five phenotypic factors including drive (urge or craving to smoke), priority (valuing smoking over other reinforcers by avoiding situations where smoking is not favorable), tolerance (need and ability to smoke larger quantities over time), continuity (irregular daily smoking pattern), and stereotypy (consistent and regular daily smoking pattern). The total NDSS score is positively associated with the FTQ, the FTND, quantity smoked per day, difficulty quitting smoking, withdrawal severity, and DSM-IV nicotine dependence [19,67]. In two independent samples, the drive, priority, and tolerance factors were correlated with the FTQ and daily smoking quantity, the drive and priority factors were related to difficulty quitting, and the drive factor was further associated with withdrawal severity [67].

Another multidimensional instrument of nicotine dependence is the Modified Reasons for Smoking Scale (MRSS) which captures seven factors [68], and is an extension of the original six factor Reasons for Smoking Scale (RSS) [69] developed to assess smoking motives. The MRSS factors include addictive smoking (cravings to smoke), smoking because of the pleasurable effects, smoking for tension reduction/relaxation, social smoking, stimulation smoking (smoking for the activating effects), habit/automatism (smoking without thinking), and handling (smoking for the ritualized behaviors). The addictive factor score was associated with increased risk in FTND nicotine dependence, and higher scores on both the addictive and on the habit/automatism factors were associated with shorter time to the first cigarette after waking [68]. Higher scores on the habit/automatism factor were also associated with cessation failure and with negative affect in women only [68]. The addictive factor from the RSS correlates with the NDSS total scale score and with NDSS drive and priority factors, and the RSS automatic factor correlates with NDSS total scale score and NDSS priority, tolerance, and stereotopy factors [67].

A third recently developed multidimensional instrument is the Wisconsin Inventory of Smoking Dependence Motives (WISDM-68), which captures thirteen factors [70]. These factors include smoking because of emotional attachment to smoking, automatic smoking, smoking for cognitive enhancement, craving/urges to smoke, smoking in response to cue exposure, loss of control over use, positive reinforcement, negative reinforcement, social smoking, taste and sensory properties of smoking, smoking for weight control, and tolerance. All but the social smoking factor were significantly related to the FTND with the highest correlation for the tolerance factor (0.78) and the lowest for the weight control factor (0.11) [70].

The significant but imperfect correlations in dimensions across instruments suggest overlap but also specificity to the measurement of nicotine dependence. Taken together, what does the multifactorial nature of nicotine dependence tell us, and how do we proceed in applying this knowledge to genetic research and to practical public health issues? First, understanding the etiology of nicotine dependence dimensions in relation to earlier stages of smoking, in relation to quitting, and in relation to comorbid substance use (e.g., alcohol) and psychopathology (e.g., attention deficit hyperactivity disorder) could reveal subgroups of smokers that may benefit from different interventions and treatment. Second, investigation of the relative contribution of genetic and environmental influences on the etiology of nicotine dependence can reveal the degree of shared and specific effects that might facilitate both gene discovery and identification of environmental factors that modify genetic effects.

Ideally, as scales that are more specific to nicotine dependence emerge, phenotypic stability, examination of reliability and validity, and replicability across populations should be encouraged in order to converge on a systematic

approach to the characterization of nicotine dependence. Currently, there are no measures of nicotine dependence that incorporate measurement of underlying neuropathological processes nor are there any measures that determine the ''severity'' of nicotine dependence.

4. Genetics of nicotine dependence and smoking cessation

4.1. *Neurobiology of nicotine dependence*

We begin with a brief and simplified view of the biological basis of nicotine dependence, to help inform the reader on the choice of candidate genes in measured genetic studies of nicotine dependence and smoking cessation.

Upon inhalation of cigarette smoke, nicotine quickly crosses the blood brain barrier and binds to nicotinic acetylcholine receptors (nAChRs) in the brain [71]. Activation of nAChRs stimulates the mesocorticolimbic dopamine system (i.e., reward pathway) to produce the primary reinforcing effects of nicotine [72]. Stimulation of dopamine neurons in the ventral tegmental area (VTA) by nicotine via high affinity $\alpha4\beta2$ nAChRs (and by all drugs of abuse via specific receptor targets) causes increased firing in terminal dopaminergic fields, such as the nucleus accumbens, amygdala, and the prefrontal cortex. Activation of dopaminergic VTA neurons is also mediated by excitatory glutamatergic neurons projecting primarily from the prefrontal cortex [73]. Presynaptic $\alpha7$ nAChRs located on glutamatergic projections enhance excitatory input [74]. Gamma-amino buteric acid (GABA) interneurons in the VTA, which also express nAChRs, and GABA-ergic projections from the nucleus accumbens to the VTA [75,76] mediate inhibitory and control processes of dopamine stimulation. Thus, the overall effect of nicotine in the VTA is via interactions of upstream and downstream effects [77]. Repeated exposure to nicotine in conjunction with environmental cues [78], causes lasting changes in dopaminergic function, which contribute to maintenance of smoking and the experience of withdrawal symptoms upon cessation [79,80].

In animal studies, disruption of dopaminergic activity via pharmacological blockade of dopamine receptors or chemical lesions of dopaminergic neurons leads to decreased nicotine-induced reinforcement [81,82], suggesting a mediating role of these receptors in the reinforcing properties of nicotine. Similarly, disruption of nAChRs by pharmacological blockade or knock-out models also contributes to decreased nicotine reinforcement [83,84]. The dopamine reward pathway is a common mechanism of addiction vulnerability to a variety of drugs of abuse [85]. Thus, while nAChRs are primary targets of nicotine, nicotine exerts direct and indirect effects on other receptor systems (e.g., opioid, serotonergic, glutamatergic) that also mediate nicotine-induced behavioral and neural changes in animal models and in humans. Variation in the genes that code for the drug receptor proteins or that code for metabolic and catabolic enzymes that influence neurotransmitter levels, represent candidate genes for measured genetic studies of nicotine dependence and treatment.

4.2. *Univariate investigation*

Twin studies show that significant proportion of the phenotypic variance in nicotine dependence is attributable to additive genetic effects (heritability). In adults, substantial heritability has been reported for the FTND (40–75%) [19,22,26], the HSI (59–71%) [20,86,87], and for diagnostic nicotine dependence (33.0–77.0%) [18,21,24,86,88]. Diagnostic nicotine dependence is also heritable in adolescents (44%) though unlike adult studies, 37% of the variance was also explained by environmental factors common to family members (i.e., shared environmental effects) [89]. There are also strong genetic influences on component measures of the FTND, including time to first cigarette in the morning (55–68%) [20,21] and daily cigarette quantity (45.0–70.0%) [20,21,23,25,44,90–94]. Of note, low to no significant heritability for the FTND and the FTQ have also been reported (0–17.0%) [20,44]. The low heritability estimates could be due to the inclusion of only lifetime regular smokers in the dependence definition in one study [44]. Conditioning genetic and environmental estimates of dependence on regular smoking removes genetic variation associated with vulnerability to regular smoking, which results in essentially estimating dependence-specific variation. In the second study [20], it was unclear whether only regular smokers were included in the dependence definition. These results underscore the need for careful assessment of smoking history and subsequent categorization of individuals into nicotine dependence categories. Also, the clear definition of smoking groups in publications is vital to furthering our understanding of the etiology of nicotine dependence.

Each of the seven diagnostic nicotine dependence criteria have also been shown to be heritable (26–73%) [21,27,28,86] as well as individual nicotine withdrawal symptoms (9.0–53.0%) [23]. With the exception of cessation and withdrawal symptom measures, there is no evidence for a significant contribution from shared environmental effects, suggesting that the major causes of variation are genetic and individual-specific environmental factors.

An interesting difference in the genetic impact on difficulty quitting smoking and withdrawal, compared to other nicotine dependence phenotypes, is the observed sex difference in the magnitude of the relative contribution of genetic and environmental effects. There appear to be greater genetic influences on women's risk in difficulty quitting smoking (68%) compared to men (54%), for whom shared environmental factors (26%) also appear to play a role [21]. It is possible that greater genetic risk for difficulty quitting in women contributes to the reported lower long-term cessation rates in women compared to men [95–100], though equivalent cessation rates for women and men have also been reported [36]. Experiencing depressed mood upon nicotine withdrawal has been found to be less genetically influenced in women (29%) compared to men (53%) with no evidence for a significant contribution of shared environment for either sex [23]. Considering that the reported prevalence of the depressed mood withdrawal symptom is lower in men (18.7%) compared to women (27%) [23], it could be that men who report this symptom have higher genetic risk for depressed mood compared to women. For both the difficulty quitting and depressed mood during withdrawal measures, higher genetic

risk is seen for the group that has lower prevalence of the behavior (difficulty quitting for women and depressed mood during nicotine withdrawal for men), consistent with the idea that extreme and low prevalence phenotypes may be more genetically informative [42,101].

To the best of our knowledge, there are two published reports on the heritability of multidimensional nicotine dependence constructs. One twin study using the NDSS [19] found that a three-factor structure fit the data better than the originally proposed five-factor structure [67]. The total NDSS score was moderately heritable (30%) as were the scores for the tolerance (39%) and the stereotypy/continuity (44%) factors, and there was no evidence for a significant contribution from shared environmental sources [19]. There was no evidence for a significant contribution from genetic sources on the scores from the third drive/priority factor, which was modestly influenced by shared environmental factors in men (22%) and no familial sources of variance (genetic or shared environment) in women [19]. The common feature in the heritable tolerance and continuity/stereotypy factors were items related to quantity smoked. The non-genetic drive/priority factor reflected items related to negative reinforcement (withdrawal relief) and avoiding non-smoking situations (restaurants, plane travel, and company of non-smoking friends). We have found that the DSM-IV criterion of giving up or reducing important activities in order to smoke, which is similar to the idea of avoiding non-smoking situations, was the least heritable (26%) of the seven DSM-IV criteria (39–73%), was the least commonly endorsed (6% vs. 18.3–53% for the other criteria), and contributed to a decrement in factor internal consistency [21]. Another study examined the heritability of four smoking motives considered to capture a pharmacological dimension of smoking – smoking for sedative effects (under stress and to calm down), for stimulatory effects (to get a lift), for the addictive properties (urges to smoke), and automatic smoking (smoking without remembering doing so) [102]. The study showed that the four factors were under the influence of the same genetic source, suggesting that they act through the same pharmacological mechanism [102]. In this common factor model, estimates of the genetic and environmental contribution to variation in individual factors was as follows: the automatic smoking factor was influenced by a similar degree of genetic variance in women (18%) and men (23%) with significantly greater influence of shared environmental effects in women (23%) compared to men (5%); the addictive factor was influenced to a similar degree by genetic (24% in women and 27% in men) and shared environmental effects (22% in women and 25% in men). The sedative factor had modest heritability (23% in women and 32% in men) and the stimulation factor had the highest heritability (49% in women and 56% in men), with no evidence for significant shared environmental effects on these factors [102]. The apparent magnitude differences in genetic and environmental contributions across factors could mean that within the same pharmacological pathway, some measures of dependence are more highly genetically determined than others.

The expression of genetic risk may depend on social or cultural smoking restrictions, such as the significantly lower prevalence of smoking among women compared to men in some societies (e.g., China and India), as well as in older US cohorts. Though not specific to nicotine dependence, one study found no birth cohort effects on the relative contribution of genes and environment to smoking initiation (lifetime ever smoking) in one Australian and two US samples, despite markedly higher smoking prevalence in younger compared to older birth cohorts, particularly in women [103]. Heritability of smoking initiation was found to be somewhat lower in Australian men (33%) than in US men (51%) [103]. A study in a large sample of California twins found no birth cohort differences in the contribution of genes and environment on smoking initiation in women, but a relatively lower heritability and significant shared environmental effects in the younger compared to the older male birth cohort; no significant differences were found in the prevalence of smoking initiation across male birth cohorts [104]. A study that compared genetic and environmental influences on smoking initiation and smoking persistence (smoking at the time of survey) between Australian, Finnish, and Swedish twins showed no significant differences in genetic influences on either phenotype by country, despite prevalence differences in both smoking phenotypes across countries [105]. Heritable influences on smoking persistence were also found to be similar across birth cohorts in California twins [104]. In Chinese male twins, heritability estimates for current (persistent) and heavy smoking were within the range of those reported for twins of Western origin [106] although the study did not directly compare genetic estimates across cultures. Taken together, results from these studies suggest that the expression of genetic risk for smoking initiation and persistence does not consistently vary across societies or birth cohorts, at least for the initiation and persistence broadly defined smoking phenotypes.

Consistent with the twin studies, family and adoption studies have found significant familial influences on nicotine dependence [107,108] and quantity smoked [43,109–112]. The long-standing limitation with family-based studies is the inability to separate genetic from shared environmental effects, and while twin pairs reared apart provide a very strong test of the genetic versus environmental influences, these tend to be highly selected and rare.

4.3. *Multivariate investigation – smoking stages and comorbidity*

Using twins, it is possible to understand the extent to which shared genetic and environmental factors underlie covariation between earlier and later stages of smoking as well as covariation between nicotine dependence and comorbid substance use and psychopathology. Ability to partition total covariation into common and specific genetic and environmental sources can provide an important framework for thinking about effect sizes and impact of measured genetic and measured environmental factors.

4.3.1. *Smoking stages*

Early work has shown that some of the genes that predispose to smoking initiation also predispose to smoking persistence [3,113,114]. However, the small to moderate overlap in genetic risk (0–43%) between initiation and persistence, which varies across sex, age cohort, and country of origin [113,114] suggests

that a large portion of the genetic variance is specific to each phenotype. The overlap in individual-specific environmental risk is also small (3–30%). Overall, these results suggest that liability to ever smoking is largely independent from liability to continuing to smoke. This could be due to the fact that while over 80% of individuals in some studies report that they have tried smoking cigarettes, only about one third of them will smoke beyond experimentation. Thus, the definition of ever smoking includes individuals at varying genetic and environmental risk for smoking.

Bivariate models of regular smoking and nicotine dependence show substantial genetic overlap between these smoking stages [18,115]. One of these studies estimated that 31% of the total genetic variance on nicotine dependence defined as a factor score of FTQ and some DSM-IV items was specific to dependence and was not shared with regular smoking [115]. Trivariate models examining genetic and environmental influences on the transition from smoking initiation (ever smoked cigarette(s)) to regular smoking (ever smoked at least 7 days per week for at least 4 weeks) to nicotine dependence show larger overlap in liability to smoking initiation and regular smoking, and a smaller shared liability between regular smoking and FTND nicotine dependence [22,116]. After accounting for the genetic effects on earlier stages, 17–21% of the total genetic variance on regular smoking and 23–24% of the total genetic variance on nicotine dependence were found to be specific to these phenotypes. Unlike earlier studies, these results suggest that the majority of the genetic vulnerability to later smoking stages is in common with that of earlier stages. Differences in results may be related to differences in phenotypic definitions and refinement of multivariate twin models.

In relation to smoking cessation phenotypes, difficulty quitting smoking and nicotine withdrawal appear to have common genetic mechanisms [27,28], which may help explain why individuals who experience higher withdrawal severity have greater difficulty remaining abstinent from cigarettes [27,28]. In other words, some of the genes that predispose to difficulty quitting smoking cigarettes also predispose to greater withdrawal severity. Further, nicotine withdrawal shares genetic risk not only with failed cessation but also with quantity smoked [23] as well as with earlier stages of smoking such as smoking progression beyond experimentation [23]. These results suggest that once genetic risk for cigarette smoking is expressed it may contribute to a lifetime course of cigarette smoking that leads to dependence and to cessation difficulties. At the same time, however, up to 23% of the total variance in nicotine withdrawal was genetic variance specific to this later stage of nicotine withdrawal, suggesting that different genetic factors are associated with nicotine withdrawal, even after controlling for beginning to smoke and quantity smoked.

Taken together, these studies suggest that genetic factors that predispose individuals to starting to smoke also confer liability to later smoking stages, including nicotine withdrawal. However, there is a substantial proportion of genetic variation that is specific to later smoking stages. In essence, stage models in twins characterize the dynamic changes in genetic and environmental sources of variation and covariation on the natural history of smoking behavior.

4.3.2. *Comorbidity*

Nicotine dependence co-occurs with a range of other substances and mental conditions [111,117–123]. Investigation into the possibility that shared familial factors may explain smoking-alcohol use comorbidity has by far yielded the most results to date. Twin and family studies show that covariation between smoking and alcohol drinking and between nicotine and alcohol dependence is in part due to overlapping familial (genetic and shared environmental) risk factors [24,44,90–92,111,124–126]. Additionally, linkage studies show the existence of susceptibility loci for both tobacco and alcohol use on chromosomes 1, 2, and 4 [127–132]. A genomewide association study focused on alcohol dependence and habitual smoking comorbidity identified 8 candidate genes located on chromosomes 1, 3, 7, 9, 10, 12, and the X chromosome that may predispose to vulnerability to the use of both substances [133]. Methods and use of linkage and gene association studies are explained in the next two sections.

Taking advantage of convergent evidence for common genetic susceptibility to tobacco and alcohol use, one study examined the association of the α4 and β2 high affinity neuronal nicotinic acetylcholine receptor (nAChR) genes (*CHRNA4* and *CHNRB2*) with a range of cigarette and alcohol use phenotypes [134]. The study found consistency in the association of one *CHNRB2* SNP and adverse subjective reactions to both alcohol and cigarettes in both Caucasians and Hispanic samples. While the study did not correct for multiple comparisons, the area of chromosome 1 where *CHNRB2* is located has been repeatedly implicated in tobacco and alcohol comorbidity. Investigation of shared genetic risk between smoking and alcohol use provides a concrete example of how convergent evidence from neurobiologic, pharmacologic, epidemiologic, twin, linkage, and gene association studies has identified at least one candidate gene that may play a role in genetic susceptibility to two complex disorders.

A recent study combined within and across substance stage models to simultaneously examine the genetic and environmental relationship between smoking and cannabis use initiation and progression to regular use [116]. The study found common genetic liability to smoking and cannabis use initiation and to smoking and cannabis use progression. Liability to initiation of one substance was not related to progression on the other substance [116].

Genetic studies on nicotine dependence and comorbid psychopathology are few. Modest familial and genetic association has been reported between different measures of depression and cigarette smoking [135–138], suggesting that the observed phenotypic covariation is largely attributable to sources other than shared genetic and environmental liability. One study in male Vietnam veteran twins found that 62% of the phenotypic correlation between post-traumatic stress disorder and nicotine dependence was attributable to shared genetic risk [88]. The strong relationship between schizophrenia and smoking is only slightly attributable to common familial vulnerability [139], suggesting largely independent factors that confer vulnerability to each condition. A significant association between nicotine dependence and eating disorders also could not be explained by shared familial risk [122].

While the literature on nicotine dependence and comorbid conditions in genetically informative samples is surprisingly scant, two important messages can be deduced from what we see so far. First, to the degree that shared genetic or environmental factors explain some of the observed covariation, a larger proportion of the covariation remains unexplained by these shared familial factors, suggesting that nicotine dependence and comorbid disorders need to be understood both in terms of their overlap and their specificity. Second, evidence for specificity in the familial transmission of nicotine and alcohol dependence, and of smoking and cannabis use initiation and progression suggest that substance use cannot be viewed as a single overarching disorder, and there is need for diagnostic specificity of substance use, as may be available in the next (5th) edition of the DSM (DSM-V).

4.4. *Linkage studies of nicotine dependence*

Linkage studies compare the phenotypic resemblance between family members (sibling pairs or parent-offspring pairs) to the probability that they share allelic variation across the genome. Where allele sharing is greater than that expected by chance alone, an association between that chromosomal region and the phenotype is expressed as a linkage peak with a magnitude expressed as a LOD score (logarithm of the odds of observed over expected linkage). Linkage analysis is an exploratory technique. It does not identify specific genes but identifies genomic regions that may contain candidate genes for the trait of interest. We summarize linkage results for nicotine dependence and smoking cessation-related phenotypes (as opposed to more broadly defined smoking phenotypes such as ever smoking) that have been reported with high confidence; that is, results that have met genome-wide significance levels (i.e., through simulation analysis, which accounts for multiple testing at many different loci by identifying linkage peaks with LOD scores of sufficient magnitude as to minimize false positive results per study) or results that were replicated across different methods of analysis or across independent samples.

Significant linkage for the FTQ has been reported on chromosome 2 [31], and for the FTND, on chromosomes 5 [29] and 6 [32]. Quantity smoked has been more commonly examined in linkage studies with significant linkage reported for chromosomes 1, 4 [140], 6 [141], 9 [127,142], 10 [30], 11 [141–143], and 22 [144]. Implication of multiple genomic loci on the same quantity phenotype speaks to trait heterogeneity supporting twin findings where heritability magnitude reflects the influences of the additive effects of many genes.

To the best of our knowledge, only one linkage study has reported on results relating to smoking cessation. This study found that a summary measure of nicotine withdrawal symptoms and lifetime short term quit attempt (more than 1 month but less than 1 year) showed suggestive linkage to the same but not completely overlapping area of chromosome 6 as did the total FTND score [32]. While gene association approaches may provide more statistical power, a family-based linkage approach continues to be important to identify genomic regions of interest, particularly within different ethnic cultures, and may continue to be the best way to detect rare genetic variants [145].

With advances in nicotine dependence assessment, statistical genetic methodology, molecular genetics (e.g., increased density of genomic markers), and increasingly larger and more highly statistically powered studies, we expect the number of reports of linkage results for nicotine dependence and smoking cessation to increase substantially over the next year or two. These advances will circumvent some of the pitfalls associated with linkage studies to date, such as relatively small samples, broadly defined phenotypes, insufficient density of genomic markers, and population stratification (i.e., differences in genetic marker frequencies across ethnic groups). Specificity, both in terms of phenotype definition and location of a linkage signal is vital for complex disorders such as nicotine dependence, where total genetic variation is attributed to the effects of many genes of small effects found throughout the genome, as opposed to a few genes of large effects.

4.5. *Candidate genes for nicotine dependence*

4.5.1. *Gene association studies*

Gene association studies test the relationship between variation in candidate genes and nicotine dependence. Candidate genes can be identified based on their location within linkage peak support intervals and based on knowledge of the neural mechanisms that mediate and maintain smoking behavior. More recently, with technological innovations in simultaneous screening for variation in thousands of genes, exploratory genomewide association analyses have also been conducted. Genetic variation is marked by variable number of tandem repeats (VNTRs), gene insertions/deletions, or more often, single nucleotide polymorphisms (SNPs), which represent a change in a single DNA base that differs from the usual DNA base at the same position. The next few paragraphs summarize gene association studies of nicotine dependence phenotypes. Except where explicitly stated, it is important to note that the functional significance of variation in the reported candidate genes is not known at this time. We also limit our summary to reports that adjusted for multiple testing, underwent permutation analysis, or presented novel methods of analysis.

Because of the central role of the dopaminergic system in substance use reward pathways, dopaminergic genes are often prioritized for analysis. A family study of opioid or cocaine dependent sib-pairs examined association of DSM-IV and FTND nicotine dependence in European Americans and African Americans with variation in the D2 dopamine receptor gene (*DRD2*) and three other genes located in the same region of chromosome 11q23 (ankyrin repeat and kinase domain containing 1 gene, *ANKK1* whose protein product is involved in signal transduction; neural cell adhesion molecule gene *NCAM1* whose protein product is involved in cell-cell interaction; and tetratricopeptide repeat domain 12 gene, *TTC12* whose protein product may have transport function) [66]. Variation in *TTC12* and *ANKK1* genes was significantly associated with DSM-IV nicotine dependence in both ethnic groups, but no significant association was found for the *DRD2* or *NCAM1* genes [66]. The DRD2 receptor mediates dopamine transmission in brain reward circuits. *DRD2* SNPs that were tested included the A1 allele of the *Taq1* polymorphic locus of

the *DRD2* gene, which has been associated with decreased dopamine binding sites [146]. Recently, it has been shown that this locus is located in the *ANKK1* gene [147].In the same sample, variation in the dopa decarboxylase gene *DDC*, on chromosome 7p11, whose protein product converts dopa to dopamine, was associated with DSM-IV nicotine dependence in African Americans and with FTND in both ethnic groups [148]. The *DDC* gene was also significantly associated with the FTND as well as with quantity smoked and with the HSI in African Americans in an independent sample, the Mid-South Tobacco Family study (MSTF) [149]. In the MSTF study, a functional polymorphism in the catechol-*O*-methyltransferase gene (*COMT*; chromosome 22q11) involved in dopamine degradation, was associated with smoking quantity, HSI, and FTND in European Americans and African Americans with the low activity *Met* allele conferring greater risk for nicotine dependence compared to the high activity *Val* allele [150]. A gene involved in dopamine signaling, the phosphatase 1 regulatory subunit 1B (*PPP1R1B* on chromosome 17q12) was associated with smoking quantity in European Americans [151].

The second set of prioritized candidate genes for nicotine dependence is the nAChRs because they are direct targets of nicotine. In the MSTF study, variation in the high affinity α4 but not the high affinity β2 nAChR subunits (*CHRNA4* gene on chromosome 20q13 and *CHRNB2* gene on chromosome 1q21) was associated with smoking quantity and the FTND in European Americans and with the HSI and FTND in African Americans [152]. In animal studies, both the α4 and β2 nAChR subunits appear to mediate nicotine reward [83,84]. Variation in the β1 nAChR subunit (*CHRNB1* gene on chromosome 17p13) was associated with smoking quantity, the HSI, and the FTND in both ethnic groups [153]. In the same region of chromosome 17p13, there was significant association of variation in the $GABA_A$ receptor associated protein gene (*GABARAP*) and the discs large homolog 4 gene (*DLG4*) with smoking quantity and the FTND in European Americans but not African Americans [34]. Variation in the gene coding for the M1 muscarinic acetylcholine receptor (*CHRM1*, chromosome 11q13), the second major type of acetylcholine receptor found in both the brain and the periphery, was associated with smoking quantity and FTND in African Americans but not European Americans [153].

The μ-opioid receptor is of interest because of its role in substance use and dependence across several drug classes [154]. In a sample of unrelated individuals recruited from a twin study, one SNP (rs10485057) and a three SNP haplotype block within the μ-opioid receptor gene (*OPRM1* on chromosome 6q24–25) were associated with FTQ nicotine dependence [30]. However, the most widely tested functional SNP in this gene, rs1799971 (A118G), results in increased affinity of the μ-opioid receptor for its endogenous ligand β-endorphin which, in turn, is associated with elevated pain threshold in humans [155], was not associated with nicotine dependence.

In the MSTF study, other genes located in candidate linkage peak regions include (1) a cluster of genes on chromosome 9q22; significant association of the $GABA_B$ receptor subunit 2 gene (*GABAB2*), the tyrosine kinase receptor gene (*NTRK2*), and the Src homology 2 domain-containing transforming protein C gene (*SHC3*) was found with smoking quantity, HSI, and FTND measures in both European Americans and African Americans [156–158]; and (2) a haplotype in the brain derived neurotrophic factor gene (*BDNF*; chromosome 11p13) was significantly associated with nicotine dependence in European American males [159].

In relation to observed differences in gene associations across ethnic groups, it is notable that but for one study [66], different combinations of SNP haplotypes were associated with nicotine dependence measures in European Americans compared to African Americans even in studies that reported significant associations in both ethnic groups [150,152,153,158,159]. These results suggest differences in the genetic mechanisms for nicotine dependence across ethnicity and provide rationale for careful examination of population stratification in linkage and gene association studies, as mentioned earlier.

4.5.2. *Genomewide association studies*

Genomewide association analysis is an exploratory approach to identifying potentially important genes for complex behavior. A high-density genomewide association study of FTND-defined nicotine dependence identified 35 significant SNPs across the genome that marked 14 genes [33]. With exception of the gene coding for the β3 nAChR subunit (*CHRNB3*), which was expected to be associated with nicotine dependence, the majority of the identified candidate genes were novel. These genes were associated with several biological processes including cell adhesion (*CTNNA3, AGER,* and *NRXN1*), lipid metabolism (*AGPAT1* and *FTO*), signal transduction (*GPSM3*), protein transport (*VPS13A* and *CLCA1*), ion transport (*TRPC7*), regulation of transcription (*PBX2*) and developmental processes (*NOTCH4*), and protein catabolism (*RNF5* and *FBXL17*). While none of these 35 SNPs remained statistically significant after correction for multiple testing, the study is important in its technological achievement and its nomination of novel candidate genes [33]. In the same sample, a detailed investigation of variation in genes coding for nAChRs identified a significant role for the α5 (*CHRNA5*) and β3 (*CHRNB3*) subunits in nicotine dependence [35]. These analyses also found strong association between the FTND and variation in the potassium ion channel *GIRK2* involved in action potential propagation and the α4 subunit of the $GABA_A$ receptor complex (*GABRA4*) involved in inhibitory neuronal signaling. Most prior focus has been on the α4 and β2 nAChR subunits because they are widely expressed in the brain and have high affinity for nicotine [160].

A second genomewide association study has also nominated nicotine dependence candidate genes in cell adhesion, signal transduction, and transport functions [161]. In addition, this study compared successful quitters versus non-quitters and found candidate genes involved in functions similar to those for nicotine dependence, but also a number of genes involved in Mendelian disorders and in regulation of transcription and translation [161]. Thus, despite differences in samples and methods, there is convergence in the importance of several neural systems, such as cell adhesion and signal transduction, in the neurobiology of nicotine dependence.

Many issues surround the synthesis of the data stemming from numerous gene association findings [162] including (1) the need for replication; (2) the fact that negative findings are

meaningless in underpowered samples; (3) the importance of thoughtful definition of cases and controls; and (4) the necessity of examining linkage disequilibrium (LD) or associations between SNPs/genes that reside near one another on the same chromosome. A prime example is the *DRD2/ANKK1* genes which are in close physical proximity to one another, where it is unclear what gene or combination of genes in LD (haplotype blocks) might be accounting for the observed associations [147]. Phenotypic assessment should also include environmental variables potentially associated with smoking-related behavior in order to examine important gene by environment interactions. Genomewide association studies wrestle with the problem of multiple testing (inflated false positive rates) and there currently is no accepted approach for testing the combined effects of thousands of SNPs (or hundreds of genes), in order to ultimately assess the additive genetic effects (and gene by gene interaction) on the trait of interest. Future work might begin to evaluate the total amount of phenotypic variance in smoking-related behavior that can be accounted for by SNPs and the degree to which these estimates converge with findings from twin studies. There will be the additional challenge of how to interpret simultaneous influences of genes that confer risk or protection to nicotine dependence. It is important to keep in mind that gene association studies do not imply any causal influences on behavior; they simply show a relationship between genetic variation and lifetime smoking risk.

5. Genetics of pharmacotherapy for nicotine dependence

Substantial variability in response to pharmacological treatment for nicotine dependence has lead to investigation of how genetic variation may contribute to this variability [163]. Until recently, nicotine replacement therapy (NRT) and the antidepressant bupropion represented the only forms of approved pharmacological treatment for nicotine dependence [164]. Some of the genes initially examined in NRT and bupropion clinical trials were dopaminergic genes because of the key role of dopamine in substance abuse. Three functional polymorphisms of *DRD2* associated with decreased protein function have been investigated in relation to treatment efficacy. An insertion/deletion polymorphism (−141C *Ins/Del*) is located in the promoter region of the *DRD2* gene [165]. The low activity allele (−141C *Del*) was associated with better response to placebo, better response to NRT treatment, and worse response to bupropion [166]. NRT efficacy was increased with the combination of the −141C *Del* and variation in the neuronal calcium sensor-1 protein (*FREQ*) which regulates *DRD2* function [167], indicating the potential importance of gene–gene interaction effects on treatment efficacy. For a second SNP located in exon 7 of the *DRD2* gene (C957T) [168], the variant T allele was associated with better cessation rates overall but it had no effect on NRT or bupropion treatment efficacy [166]. The *Taq1* A1 allele, associated with decreased DRD2 binding, was associated with greater NRT effectiveness in one study [169], but more commonly, it has been shown to have no effect on treatment response in clinical trials [170–173]. The effect of this polymorphism on abstinence appears to be exacerbated in combination with variation in the dopamine beta hydroxylase gene (*DBH*) [169] or the dopamine transporter gene (*SLC6A3*). The DBH enzyme catalyzes the conversion of dopamine to norepinephrine; the dopamine transporter (DAT) is involved in reuptake of dopamine from the synapse into the presynaptic terminal.

To the best of our knowledge, the only other dopamine system receptor that has been investigated in clinical trials is the dopamine D4 receptor (*DRD4*). Two functional *DRD4* polymorphisms associated with decreased protein function were investigated: a VNTR in exon 3 (long vs. short repeat allele) and a SNP in the *DRD4* promoter (C-521T). The long risk allele of the VNTR polymorphism was associated with worse response to placebo and NRT, and no effect was found for the promoter polymorphism [37].

Variation in other dopamine system genes in relation to pharmacotherapy response include the dopamine transporter gene *SCL6A2* and the catabolic dopamine enzyme gene *COMT*. The 9-repeat variant allele of the *SLC6A3* gene associated with increased transporter availability [174] has been associated with better response to NRT or bupropion [175]. The common Val108/158Met functional polymorphism in the *COMT* gene leads to reduced COMT activity. The low activity *Met* allele was associated with better response to NRT in women but not in men [176] and no significant effect on response to bupropion in Caucasian or African American women [36].

In relation to genes other than those directly in the dopaminergic pathway, the possible effect of variation in the serotonin transporter gene *5HTTLPR* has been examined in smoking cessation trials because the serotonin transporter is a major target for selective serotonin reuptake inhibitors, and antidepressant treatment for smoking cessation has been useful in increasing abstinence rates [177]. To date, variation in *5HTTLPR* has not been significantly associated with response to placebo, NRT, or bupropion [178,179].

There is evidence for association between the A118G functional polymorphism in the μ-opioid receptor gene (*OPRM1*) and treatment response to NRT [39,100]. However, the direction of the effect varied across these two studies, possibly due to ancestral and/or gender differences.

An interesting line of research addresses the question of whether nicotine metabolism contributes to nicotine dependence. Slow nicotine metabolizers may smoke fewer cigarettes because of the higher nicotine concentration in their plasma compared to fast (wild type) metabolizers [180,181] and may progress to nicotine dependence more slowly [182] though no relationship between rate of metabolism and nicotine dependence has also been reported [183]. The gene that catalizes 80% of the conversion of nicotine to its proximal metabolite cotinine and that further metabolizes cotinine to *trans*-3′-hydroxycotinine is the liver enzyme cytochrome P450 A6 (*CYP2A6*). Variant alleles of the *CYP2A6* gene are associated with slower nicotine metabolism [183]. We are not aware of published research on the effect of *CYP2A6* variation on treatment response in clinical studies. However, one study showed that lower metabolic ratio of *trans*-3′-hydroxycotine to cotinine, an indicator of *in vivo* CYP2A6 activity, was associated with higher NRT (nicotine patch) efficacy [184] suggesting that higher plasma concentrations of nicotine as a result of patch treatment help maintain smoking abstinence.

A second line of metabolism research involves another cytochrome P450 liver enzyme, CYP2B6, which metabolizes bupropion. There was a significant therapeutic effect of bupropion in the slow metabolizer group [185], however this effect was driven by their significantly worse response to placebo compared to the wild type metabolizers. In the wild type *CYP2B6* group, there was no difference in abstinence rates between placebo and bupropion treatment and these rates were the same as abstinence in response to bupropion in the variant genotype group. Thus, it appears that slow metabolic gene variants contribute to better therapeutic response, however this effect may be driven by genotype effect on placebo response and not on active treatment response.

In May 2006, the Food and Drug Administration approved varenicline for nicotine dependence treatment. Varenicline is a partial agonist at $\alpha4\beta2$ nAChRs [14] and is 2 to 3 times more effective than NRT or bupropion in smoking cessation trials [15–17,186–188], possibly due to the specificity of its target. Identification of functional polymorphisms with reasonable minor allele frequencies (e.g., 10% or higher) in *CHRNA4* and/or *CHRNB2* genes might present a future avenue for research examining the pharmacogenetic effects of varenicline.

The pharmacogenetic research field recognizes the inherent limitations in examining a handful of genes using fairly small sample sizes. Results may vary by sex, by racial/ethnic distribution of the sample, and as a function of differences in allele frequencies of wild type and variant genotypes [36,170,176]. In addition, the significant pharmacogenetic effects rarely persist longer than 1 week after end of treatment, which does not adequately address the goal to increase the prevalence of long-term smoking cessation. As discussed in relation to the nicotine dependence phenotype, variation in the outcome phenotype examined in pharmacogenetic studies can also influence pharmacogenetic results [189]. Clearly, genetic susceptibility is but one aspect of a multivariate problem. All smoking cessation trials include behavioral counseling for all treatment conditions. Behavioral counseling is an environmental intervention that may interact with genotype. The effect of environmental factors, the interaction of these factors with genetic variation, the interaction between genetic factors, and the influence of comorbid conditions represent next important steps in pharmacogenetic smoking cessation trials, which will require large sample sizes and collaborative efforts. Few studies to date have actually been designed specifically to test pharamcogenetic effects in the context of smoking cessation treatment [190], such as randomizing by genotype.

Single gene results, although significant, likely account for only small amounts of total phenotypic variance. Simultaneous investigation of the effects of many genes would provide a more comprehensive and informative approach to understanding pharmacogenetic results. The use of twins in pharmacogenetic trials could be informative in estimation of the degree of total genetic variation accounted for by single gene polymorphisms. As other reviews have clearly noted [190–193], the field of pharmacogenetics as applied to smoking cessation is in the very early stages and conclusions based on recent findings should be tempered with the understanding that replication and additional laboratory work are needed, that effect sizes are small, that nicotine dependence is a complex behavior with multiple genetic and environmental underpinnings, and that ethical and social issues are of paramount importance. Collaborative input from diverse disciplines can enrich both the etiologic focus of the science and provide the groundwork for potential future treatment applications.

6. Emergent issues in the genetics of nicotine dependence and treatment

6.1. The role of the environment

The role of measured environmental factors on genetic susceptibility can be examined in twin studies by investigating how heritability magnitude changes across different levels of an environmental factor (i.e., gene by environment interaction). Twin studies have shown that inherited liability to smoking initiation [194,195], alcohol use [196–200], depression [201], and disinhibition [202] was more pronounced in what may be considered higher risk environments, such as being single compared to being married (or living together) [197,201], in urban compared to rural settings [200], at lower levels of socioeconomic status [196], at lower religiosity [195,198,202], with low parental monitoring [194], and low parental closeness [199]. These studies suggest that a protective environment moderates (reduces) inherited risk to the examined substance use and psychopathology measures. Alternatively, heritability of antisocial behavior [203] and cognitive aptitude in children and adolescents [204,205] was higher in advantaged socioeconomic environments, suggesting that for these behaviors a protective environment is permissive for expression of heritable influences.

The moderating effect of environmental factors on the relationship between variation in single genes and behavioral traits is a topic that has gained significant ground since the first publication on this topic. It has been reported that the short promoter allele of the serotonin transporter gene (*5-HTTLRP*), which is associated with lower efficiency of transporter function, significantly predicted depression pathology in high stress, but not in low stress environments [206]. Some subsequent studies have replicated these results [207–209], but others have not [210–212]. One study conducted in young women found a significant interaction between one SNP in the serotonin receptor-6 gene (*HTR6*; C276T) and traumatic life experience that predicted smoking initiation, but not nicotine dependence [213]. Specifically, individuals homozygous for the C allele were more likely to initiate smoking if they had also experienced trauma, compared to those who had not experienced trauma. The significant interaction was found just for one of five serotonin receptor genes that were investigated, as well as the serotonin transporter gene [213]. Evidence for an interaction between measured genetic and environmental factors could provide more specific targets for prevention and intervention programs.

6.2. Genetics of tobacco use other than cigarettes

This review focused on nicotine dependence and smoking cessation treatment as they relate to cigarette smoking

behavior. However, a brief summary of the genetics of other forms of tobacco is warranted. To the best of our knowledge, there is a single study that specifically estimated the relative contribution of genetic and environmental factors on the daily amount of cigarettes, cigars, pipes, dip (moist snuff), and chewing tobacco [214]. The authors found significant heritable influences on daily consumption of cigarettes (64%) and dip (43%). Modest heritable influences were found for daily consumption of chewing tobacco (19%) though this estimate was not significantly different from zero. No heritable influences were found for daily consumption of cigars or pipes, and moderate shared environmental effects were found for both (26% for cigars and 32% for pipes), suggesting some familial sources of variability. Notably, individual differences in daily consumption of cigars, pipes, and chewing tobacco were most strongly attributable to individual-specific environmental sources. However, this might have been due to low statistical power. Interestingly, the authors were unable to constrain parameter estimates across different types of tobacco, suggesting genetic and environmental specificity across modes of tobacco administration. An older study reported a higher concordance rate for current use of cigars or pipes among MZ male twin pairs (3.3) relative to DZ twin pairs (2.1), suggesting heritable influences on these behaviors [215]. However, the fact that the MZ concordance was less than twice the DZ concordance also suggests significant shared environmental effects on current cigar and pipe use.

Clearly, there is need to specifically investigate the genetics of tobacco use other than cigarettes, including dependence on and cessation from use of other forms of tobacco.

6.3. *Moving away from nicotine*

Nicotine dependence has been synonymous with tobacco addiction/dependence for many years. However, nicotine dependence is not necessarily the same as tobacco dependence given that long term quit rates even following pharmacologic treatment remains around 10% [10]. Non-nicotine factors of smoking contributing to dependence include sensory and behavioral cues, which represent dimensions of dependence in multidimensional instruments [70] as well as other pharmacologically active constituents that are present in tobacco mainstream smoke [216].

Non-nicotine smoke components such as ammonia can indirectly influence reinforcement by affecting sensory cues (i.e., altering the taste) or have a direct influence on the brain reward circuit alone or by interacting with nicotine. Compounds with monoamine oxidase (MAO) inhibitory properties have been identified in tobacco smoke [217–219]. Inhibition of MAO leads to decreased dopamine metabolism thus increasing levels of extracellular dopamine, which is thought to contribute to tobacco dependence [217,220]. Inhibition of MAO activity is not due to smoke generated by burning cigarette paper or by nicotine exposure alone at concentrations that are found in smokers' blood, indicating that other compounds in tobacco smoke contribute to decreases in MAO activity [221,222]. Pharmacological blockade of MAO activity in rats potentiates nicotine-induced locomotor sensitization [223], a process thought to parallel the addictive properties of drugs of abuse including nicotine [80,224]. Further, MAO inhibition induces nicotine self-administration, a behavior absent in rats that were not pre-treated with MAO inhibitors [225,226]. These results suggest an interaction between nicotine and MAO inhibitors found in tobacco smoke leading to the development and maintenance of smoking behavior and addiction.

Acetaldehyde, which is one of the most abundant constituents in tobacco smoke [227] has been shown to have reinforcing properties on its own in animal models [228]. Acetaldehyde can enhance the acquisition of nicotine self-administration and potentiate nicotine-induced locomotion [229]. One possible mechanism of action is that acetaldehyde may enhance the effects of stress leading to changes in nicotine-induced behaviors [230]. The formation of biologically active products such as salsolinol and harman also contribute to the reinforcing effects of acetaldehyde [228]. These two products can enhance the rewarding/addictive nature of tobacco constituents by increasing levels of monoamines such as dopamine either directly or by inhibition of MAO. Smoking is often observed concurrently with alcohol use and coffee drinking. Acetaldehyde is the first metabolite of alcohol, and harman and non-harman is present in coffee. Thus, the association between smoking and other behaviors may be due, in part, to the interaction of acetaldehyde and its metabolites with nicotine.

These additional compounds in tobacco may contribute to the addictive nature of smoking and could provide additional targets for understanding genetic and environmental influences that are associated with tobacco dependence which, in turn, could lead to more effective pharmacotherapies.

6.4. *The role of transdisciplinary and translational research*

Nicotine dependence is a complex disorder whose definition and treatment relies on evidence-based research from human and animal studies in a variety of disciplines including ethics, policy, public health, epidemiology, sociology, psychology, pharmacology, neuroscience, psychiatry, behavior genetics, molecular genetics, education, and medicine. While researchers and clinicians agree that a multifaceted, transdisciplinary, and translational approach to the understanding of nicotine dependence and treatment is desirable, the implementation of such an approach requires significant financial and human resources [87,231,232]. Additionally, it is important that research findings be adequately disseminated to clinicians, to patients, as well as to the general public [233,234]. Information can be a powerful tool, and sometimes may represent a deterrent to drug use (e.g., never trying smoking) or motivation for behavioral change (e.g., seeking help for drug abuse).

The NIH has bolstered transdisciplinarity in tobacco research through its funding of Transdisciplinary Tobacco Use Research Centers (TTURCs), which are now in the second round of 5-year funding. The current centers focus on understanding the mechanisms of treatment efficacy (University of Wisconsin), the risk factors for treatment failure (Yale University), and testing novel approaches to treatment (University of Pennsylvania); and on research on tobacco control policy (Roswell Park Cancer Institute), harm reduction (University of Minnesota), the interplay of genes and preven-

tion in multi-cultural settings (University of Southern California), and identification of more precise and informative measures for lifetime tobacco use patterns (Butler Hospital, Brown University). Together, these centers span numerous disciplines and involve coordinated efforts that are likely to substantially contribute to the field of nicotine dependence and pharmacotherapy.

7. Conclusions

The goal of this review was to provide a critically comprehensive snapshot of the progress, challenges, and future direction of the genetics of nicotine dependence and pharmacotherapy. Considering that some of the first twin pair comparisons on smoking behavior showing that smoking is in part genetically influenced date back to the early 1960s [235], one could argue that progress has been fairly slow. However, at this time, there is rapid development of molecular and statistical methods necessary to address the multifactorial interplay of genes and environment on the development and maintenance of complex behavior such as nicotine dependence. At the same time, recognition of the multidimensionality of the problem among the research community and funding agencies has resulted in transdisciplinary and translational efforts to bridge the gaps and approach the problem with multi-layered solutions. Assuming continuation of the pace of innovation in behavioral measurement, molecular methodology, and statistical analysis, we expect to see much more convergence of results in the future as well as exciting new discoveries.

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