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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.

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## 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.

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## 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].

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## 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 stereotypy 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  $\alpha 4\beta 2$  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  $\alpha 7$  nAChRs located on glutamatergic projections enhance excitatory input [74]. Gamma-aminobutyric 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  $\alpha 4$  and  $\beta 2$  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  $\alpha 4$  but not the high affinity  $\beta 2$  nAChR subunits (CHRNA4 gene on chromosome 20q13 and CHRN2 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  $\alpha 4$  and  $\beta 2$  nAChR subunits appear to mediate nicotine reward [83,84]. Variation in the  $\beta 1$  nAChR subunit (CHRN1 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<sub>A</sub> 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  $\mu$ -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  $\mu$ -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  $\mu$ -opioid receptor for its endogenous ligand  $\beta$ -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<sub>B</sub> 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  $\beta 3$  nAChR subunit (CHRN3), 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 (GPM3), 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  $\alpha 5$  (CHRNA5) and  $\beta 3$  (CHRN3) 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  $\alpha 4$  subunit of the GABA<sub>A</sub> receptor complex (GABRA4) involved in inhibitory neuronal signaling. Most prior focus has been on the  $\alpha 4$  and  $\beta 2$  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  $\mu$ -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 catalyzes 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'-hydroxycotinine 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  $\alpha 4\beta 2$  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 *CHRNA2* 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 pharmacogenetic 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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## REFERENCES

- [1] Carlsten C, Burke W. Potential for genetics to promote public health: genetics research on smoking suggests caution about expectations. *J Am Med Assoc* 2006;296:2480–2.
- [2] Merikangas KR, Risch N. Genomic priorities and public health. *Science* 2003;302:599–601.
- [3] Heath AC. Persist or quit? Testing for a genetic contribution to smoking persistence. *Acta Genet Med Gemellol (Roma)* 1990;39:447–58.
- [4] APA. Diagnostic and statistical manual of mental disorders. Washington, DC: American Psychiatric Association; 1994.
- [5] Smith SS, Fiore MC. The epidemiology of tobacco use, dependence, and cessation in the United States. *Prim Care* 1999;26:433–61.
- [6] DHHS. The health consequences of smoking: a report of the Surgeon General. Washington, DC: Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, 2004.
- [7] Murray S. A smouldering epidemic. *Can Med Assoc J* 2006;174:309–10.
- [8] CDC. Cigarette Smoking Among Adults – United States, 2003. *Morb Mortal Wkly Rep* 2005;34:509–13.
- [9] CDC. Tobacco Use Among Adults – United States, 2005. *Morb Mortal Wkly Rep* 2006;55:1145–8.
- [10] Hughes JR, Stead LF, Lancaster T. Antidepressants for smoking cessation. *Cochrane Database Syst Rev* 2007;CD000031.
- [11] Xian H, Scherrer JF, Eisen SA, Lyons MJ, Tsuang M, True WR, et al. Nicotine dependence subtypes: association with smoking history, diagnostic criteria and psychiatric disorders in 5440 regular smokers from the Vietnam Era Twin Registry. *Addict Behav* 2007;32:137–47.
- [12] John U, Meyer C, Hapke U, Rumpf HJ, Schumann A. Nicotine dependence, quit attempts, and quitting among smokers in a regional population sample from a country with a high prevalence of tobacco smoking. *Prev Med* 2004;38:350–8.
- [13] Silagy C, Lancaster T, Stead L, Mant D, Fowler G. Nicotine replacement therapy for smoking cessation. *Cochrane Database Syst Rev* 2004;CD000146.
- [14] Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem* 2005;48:3474–7.
- [15] Keating GM, Siddiqui MA. Varenicline: a review of its use as an aid to smoking cessation therapy. *CNS Drugs* 2006;20:945–60.
- [16] Tonstad S. Varenicline for smoking cessation. *Expert Rev Neurother* 2007;7:121–7.
- [17] Williams KE, Reeves KR, Billing Jr CB, Pennington AM, Gong J. A double-blind study evaluating the long-term safety of varenicline for smoking cessation. *Curr Med Res Opin* 2007;23:793–801.
- [18] Agrawal A, Heath AC, Grant JD, Pergadia ML, Statham DJ, Bucholz KK, et al. Assortative mating for cigarette smoking and for alcohol consumption in female Australian twins and their spouses. *Behav Genet* 2006;36:553–66.
- [19] Broms U, Madden PA, Heath AC, Pergadia ML, Shiffman S, Kaprio J. The Nicotine Dependence Syndrome Scale in Finnish smokers. *Drug Alcohol Depend* 2007;89:42–51.
- [20] Haberstick BC, Timberlake D, Ehringer MA, Lessem JM, Hopfer CJ, Smolen A, et al. Genes, time to first cigarette and nicotine dependence in a general population sample of young adults. *Addiction* 2007;102:655–65.
- [21] Lessov CN, Martin NG, Statham DJ, Todorov AA, Slutske WS, Bucholz KK, et al. Defining nicotine dependence for genetic research: evidence from Australian twins. *Psychol Med* 2004;34:865–79.
- [22] Maes HH, Sullivan PF, Bulik CM, Neale MC, Prescott CA, Eaves LJ, et al. A twin study of genetic and environmental influences on tobacco initiation, regular tobacco use and nicotine dependence. *Psychol Med* 2004;34:1251–61.
- [23] Pergadia ML, Heath AC, Martin NG, Madden PA. Genetic analyses of DSM-IV nicotine withdrawal in adult twins. *Psychol Med* 2006;36:963–72.
- [24] True WR, Xian H, Scherrer JF, Madden PA, Bucholz KK, Heath AC, et al. Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch Gen Psychiatry* 1999;56:655–61.
- [25] Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J. Genetic architecture of smoking behavior: a study of Finnish adult twins. *Twin Res Hum Genet* 2006;9: 64–72.
- [26] Vink JM, Willemsen G, Boomsma DI. Heritability of smoking initiation and nicotine dependence. *Behav Genet* 2005;35:397–406.



- [27] Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, et al. Latent class typology of nicotine withdrawal: genetic contributions and association with failed smoking cessation and psychiatric disorders. *Psychol Med* 2005;35:409–19.
- [28] Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, et al. The heritability of failed smoking cessation and nicotine withdrawal in twins who smoked and attempted to quit. *Nicotine Tob Res* 2003;5:245–54.
- [29] Gelernter J, Panhuysen C, Weiss R, Brady K, Poling J, Krauthammer M, et al. Genomewide linkage scan for nicotine dependence: identification of a chromosome 5 risk locus. *Biol Psychiatry* 2007;61:119–26.
- [30] Zhang L, Kendler KS, Chen X. The mu-opioid receptor gene and smoking initiation and nicotine dependence. *Behav Brain Funct* 2006;2:28.
- [31] Straub RE, Sullivan PF, Ma Y, Myakishev MV, Harris-Kerr C, Wormley B, et al. Susceptibility genes for nicotine dependence: a genome scan and followup in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. *Mol Psychiatry* 1999;4:129–44.
- [32] Swan GE, Hops H, Wilhelmson KC, Lessov-Schlaggar CN, Cheng LS, Hudmon KS, et al. A genome-wide screen for nicotine dependence susceptibility loci. *Am J Med Genet B Neuropsychiatr Genet* 2006;141:354–60.
- [33] Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007;16:24–35.
- [34] Lou XY, Ma JZ, Sun D, Payne TJ, Li MD. Fine mapping of a linkage region on chromosome 17p13 reveals that GABARAP and DLG4 are associated with vulnerability to nicotine dependence in European-Americans. *Hum Mol Genet* 2007;16:142–53.
- [35] Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 2007;16:36–49.
- [36] Berrettini WH, Wileyto EP, Epstein L, Restine S, Hawk L, Shields P, et al. Catechol-O-methyltransferase (COMT) gene variants predict response to bupropion therapy for tobacco dependence. *Biol Psychiatry* 2007;61:111–8.
- [37] David SP, Munafò MR, Murphy MF, Proctor M, Walton RT, Johnstone EC. Genetic variation in the dopamine D4 receptor (DRD4) gene and smoking cessation: follow-up of a randomised clinical trial of transdermal nicotine patch. *Pharmacogenomics J* 2007.
- [38] Lerman C, Shields PG, Wileyto EP, Audrain J, Pinto A, Hawk L, et al. Pharmacogenetic investigation of smoking cessation treatment. *Pharmacogenetics* 2002;12:627–34.
- [39] Lerman C, Wileyto EP, Patterson F, Rukstalis M, Audrain-McGovern J, Restine S, et al. The functional mu opioid receptor (OPRM1) Asn40Asp variant predicts short-term response to nicotine replacement therapy in a clinical trial. *Pharmacogenomics J* 2004;4:184–92.
- [40] Lee AM, Tyndale RF. Drugs and genotypes: how pharmacogenetic information could improve smoking cessation treatment. *J Psychopharmacol* 2006;20:7–14.
- [41] Lerman C. Helping smokers quit through pharmacogenetics. *LDI Issue Brief* 2006;11:1–4.
- [42] Rice JP, Saccone NL, Rasmussen E. Definition of the phenotype. *Adv Genet* 2001;42:69–76.
- [43] Saccone NL, Neuman RJ, Saccone SF, Rice JP. Genetic analysis of maximum cigarette-use phenotypes. *BMC Genet* 2003;4(Suppl 1):S105.
- [44] Prescott CA, Kendler KS. Genetic and environmental influences on alcohol and tobacco dependence among women. Bethesda, MD: National Institutes of Health; 1995.
- [45] DiFranza JR, Savageau JA, Fletcher K, O'Loughlin J, Pbert L, Ockene JK, et al. Symptoms of tobacco dependence after brief intermittent use: the Development and Assessment of Nicotine Dependence in Youth-2 study. *Arch Pediatr Adolescent Med* 2007;161:704–10.
- [46] DiFranza JR, Savageau JA, Rigotti NA, Fletcher K, Ockene JK, McNeill AD, et al. Development of symptoms of tobacco dependence in youths: 30 month follow up data from the DANDY study. *Tob Control* 2002;11:228–35.
- [47] Gervais A, O'Loughlin J, Meshefedjian G, Bancej C, Tremblay M. Milestones in the natural course of onset of cigarette use among adolescents. *Can Med Assoc J* 2006;175:255–61.
- [48] Fagerstrom KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addict Behav* 1978;3:235–41.
- [49] Lichtenstein E, Mermelstein RJ. Some methodological cautions in the use of the Tolerance Questionnaire. *Addict Behav* 1986;11:439–42.
- [50] Heatherton TF, Kozlowski LT, Frecker RC, Rickert W, Robinson J. Measuring the heaviness of smoking: using self-reported time to the first cigarette of the day and number of cigarettes smoked per day. *Br J Addict* 1989;84:791–9.
- [51] Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 1991;86:1119–27.
- [52] Etter JF, Duc TV, Perneger TV. Validity of the Fagerstrom test for nicotine dependence and of the Heaviness of Smoking Index among relatively light smokers. *Addiction* 1999;94:269–81.
- [53] Hudmon KS, Marks JL, Pomerleau CS, Bolt DM, Brigham J, Swan GE. A multidimensional model for characterizing tobacco dependence. *Nicotine Tob Res* 2003;5:655–64.
- [54] Chabrol H, Niezborala M, Chastan E, Montastruc JL, Mullet E. A study of the psychometric properties of the Fagerstrom Test for Nicotine Dependence. *Addict Behav* 2003;28:1441–5.
- [55] Haddock CK, Lando H, Klesges RC, Talcott GW, Renaud EA. A study of the psychometric and predictive properties of the Fagerstrom Test for Nicotine Dependence in a population of young smokers. *Nicotine Tob Res* 1999;1:59–66.
- [56] Radzius A, Gallo JJ, Epstein DH, Gorelick DA, Cadet JL, Uhl GE, et al. A factor analysis of the Fagerstrom Test for Nicotine Dependence (FTND). *Nicotine Tob Res* 2003;5:255–340.
- [57] Richardson CG, Ratner PA. A confirmatory factor analysis of the Fagerstrom Test for Nicotine Dependence. *Addict Behav* 2005;30:697–709.
- [58] Payne TJ, Smith PO, McCracken LM, McSherry WC, Antony MM. Assessing nicotine dependence: a comparison of the Fagerstrom Tolerance Questionnaire (FTQ) with the Fagerstrom Test for Nicotine Dependence (FTND) in a clinical sample. *Addict Behav* 1994;19:307–17.
- [59] Huang CL, Lin HH, Wang HH. The psychometric properties of the Chinese version of the Fagerstrom Test for Nicotine Dependence. *Addict Behav* 2006;31:2324–7.
- [60] APA. Diagnostic and statistical manual of mental disorders. Washington, DC: American Psychiatric Association; 1987.
- [61] Cottler LB, Schuckit MA, Helzer JE, Crowley T, Woody G, Nathan P, et al. The DSM-IV field trial for substance use disorders: major results. *Drug Alcohol Depend* 1995;38:59–69. discussion 71–83.

- [62] Johnson EO, Breslau N, Anthony JC. The latent dimensionality of DIS/DSM-III-R nicotine dependence: exploratory analyses. *Addiction* 1996;91:583–8.
- [63] Radzins A, Gallo J, Gorelick D, Cadet JL, Uhl G, Henningfield J, et al. Nicotine dependence criteria of the DIS and DSM-III-R: a factor analysis. *Nicotine Tob Res* 2004;6:303–8.
- [64] Moolchan ET, Radzins A, Epstein DH, Uhl G, Gorelick DA, Cadet JL, et al. The Fagerstrom Test for Nicotine Dependence and the Diagnostic Interview Schedule: do they diagnose the same smokers? *Addict Behav* 2002;27:101–13.
- [65] Hughes JR, Oliveto AH, Riggs R, Kenny M, Liguori A, Pillitteri JL, et al. Concordance of different measures of nicotine dependence: two pilot studies. *Addict Behav* 2004;29:1527–39.
- [66] Gelernter J, Yu Y, Weiss R, Brady K, Panhuysen C, Yang BZ, et al. Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. *Hum Mol Genet* 2006;15:3498–507.
- [67] Shiffman S, Waters A, Hickcox M. The nicotine dependence syndrome scale: a multidimensional measure of nicotine dependence. *Nicotine Tob Res* 2004;6:327–48.
- [68] Berlin I, Singleton EG, Pedarros AM, Lancron S, Rames A, Aubin HJ, et al. The Modified Reasons for Smoking Scale: factorial structure, gender effects and relationship with nicotine dependence and smoking cessation in French smokers. *Addiction* 2003;98:1575–83.
- [69] Russell MAH, Peto J, Patel UA. The classification of smoking by factorial structure of motives. *J R Statist Soc* 1974;137:313–46.
- [70] Piper ME, Piasecki TM, Federman EB, Bolt DM, Smith SS, Fiore MC, et al. A multiple motives approach to tobacco dependence: the Wisconsin Inventory of Smoking Dependence Motives (WISDM-68). *J Consult Clin Psychol* 2004;72:139–54.
- [71] Dani JA, Heinemann S. Molecular cellular aspects of nicotine abuse. *Neuron* 1996;16:905–8.
- [72] Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. *Eur J Pharmacol* 2000;393:295–314.
- [73] Taber MT, Das S, Fibiger HC. Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. *J Neurochem* 1995;65:1407–10.
- [74] Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 2000;27:349–57.
- [75] Walaas I, Fonnum F. Biochemical evidence for gamma-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat. *Neuroscience* 1980;5:63–72.
- [76] Kalivas PW, Churchill L, Klitenick MA. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* 1993;57:1047–60.
- [77] Mansvelder HD, De Rover M, McGehee DS, Brussaard AB. Cholinergic modulation of dopaminergic reward areas: upstream and downstream targets of nicotine addiction. *Eur J Pharmacol* 2003;480:117–23.
- [78] Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, et al. Self-administered and noncontingent nicotine enhance reinforced operant responding in rats: impact of nicotine dose and reinforcement schedule. *Psychopharmacology* 2007;190:353–62.
- [79] Miyata H, Yanagita T. Neurobiological mechanisms of nicotine craving. *Alcohol Fayetteville NY* 2001;24: 87–93.
- [80] Balfour DJ. The neurobiology of tobacco dependence: a commentary. *Respiration Int Rev Thorac Dis* 2002;69:7–11.
- [81] Corrigan WA, Coen KM. Selective dopamine antagonists reduce nicotine self-administration. *Psychopharmacology* 1991;104:171–6.
- [82] Le Foll B, Sokoloff P, Stark H, Goldberg SR. Dopamine D3 receptor ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects. *Neuropsychopharmacology* 2005;30:720–30.
- [83] Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicotine activation of alpha4\* receptors: sufficient for reward, tolerance, and sensitization. *Science* 2004;306:1029–32.
- [84] Walters CL, Brown S, Changeux JP, Martin B, Damaj MI. The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology* 2006;184:339–44.
- [85] Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci* 1999;22:521–7.
- [86] Pergadia ML, Heath AC, Agrawal A, Bucholz KK, Martin NG, Madden PA. The implications of simultaneous smoking initiation for inferences about the genetics of smoking behavior from twin data. *Behav Genet* 2006;36:567–76.
- [87] Lessov CN, Swan GE, Ring HZ, Khroyan TV, Lerman C. Genetics and drug use as a complex phenotype. *Subst Use Misuse* 2004;39:1515–69.
- [88] Koenen KC, Hitsman B, Lyons MJ, Niaura R, McCaffery J, Goldberg J, et al. A twin registry study of the relationship between posttraumatic stress disorder and nicotine dependence in men. *Arch Gen Psychiatry* 2005;62: 1258–65.
- [89] McGue M, Elkins I, Iacono WG. Genetic and environmental influences on adolescent substance use and abuse. *Am J Med Genet* 2000;96:671–7.
- [90] Hettema JM, Corey LA, Kendler KS. A multivariate genetic analysis of the use of tobacco, alcohol, and caffeine in a population based sample of male and female twins. *Drug Alcohol Depend* 1999;57:69–78.
- [91] Swan GE, Carmelli D, Cardon LR. Heavy consumption of cigarettes, alcohol and coffee in male twins. *J Stud Alcohol* 1997;58:182–90.
- [92] Swan GE, Carmelli D, Cardon LR. The consumption of tobacco, alcohol, and coffee in Caucasian male twins: a multivariate genetic analysis. *J Substance Abuse* 1996;8:19–31.
- [93] Carmelli D, Swan GE, Robinette D, Fabsitz RR. Heritability of substance use in the NAS-NRC Twin Registry. *Acta Genet Med Gemellol (Roma)* 1990;39:91–8.
- [94] Swan GE, Carmelli D, Rosenman RH, Fabsitz RR, Christian JC. Smoking and alcohol consumption in adult male twins: genetic heritability and shared environmental influences. *J Substance Abuse* 1990;2:39–50.
- [95] Osler M, Prescott E, Godtfredsen N, Hein HO, Schnohr P. Gender and determinants of smoking cessation: a longitudinal study. *Prev Med* 1999;29:57–62.
- [96] Swan GE, Jack LM, Curry S, Chorost M, Javitz H, McAfee T, et al. Bupropion SR and counseling for smoking cessation in actual practice: predictors of outcome. *Nicotine Tob Res* 2003;5:911–21.
- [97] Swan GE, Jack LM, Ward MM. Subgroups of smokers with different success rates after use of transdermal nicotine. *Addiction* 1997;92:207–17.
- [98] Ferguson JA, Patten CA, Schroeder DR, Offord KP, Eberman KM, Hurt RD. Predictors of 6-month tobacco abstinence among 1224 cigarette smokers treated for nicotine dependence. *Addict Behav* 2003;28:1203–18.
- [99] Borrelli B, Papandonatos G, Spring B, Hitsman B, Niaura R. Experimenter-defined quit dates for smoking cessation:

- adherence improves outcomes for women but not for men. *Addiction* 2004;99:378–85.
- [100] Munafò MR, Elliot KM, Murphy MF, Walton RT, Johnstone EC. Association of the mu-opioid receptor gene with smoking cessation. *Pharmacogenomics J* 2007;9:225–31.
- [101] Eaves L, Meyer J. Locating human quantitative trait loci: guidelines for the selection of sibling pairs for genotyping. *Behav Genet* 1994;24:443–55.
- [102] Gynther LM, Hewitt JK, Heath AC, Eaves LJ. Phenotypic and genetic factors in motives for smoking. *Behav Genet* 1999;29:291–302.
- [103] Heath AC, Cates R, Martin NG, Meyer J, Hewitt JK, Neale MC, et al. Genetic contribution to risk of smoking initiation: comparisons across birth cohorts and across cultures. *J Substance Abuse* 1993;5:221–46.
- [104] Hamilton AS, Lessov-Schlaggar CN, Cockburn MG, Unger JB, Cozen W, Mack TM. Gender differences in determinants of smoking initiation and persistence in California twins. *Cancer Epidemiol Biomarkers Prev* 2006;15:1189–97.
- [105] Madden PA, Pedersen NL, Kaprio J, Koskenvuo MJ, Martin NG. The epidemiology and genetics of smoking initiation and persistence: crosscultural comparisons of twin study results. *Twin Res* 2004;7:82–97.
- [106] Lessov-Schlaggar CN, Pang Z, Swan GE, Guo Q, Wang S, Cao W, et al. Heritability of cigarette smoking and alcohol use in Chinese male twins: the Qingdao twin registry. *Int J Epidemiol* 2006;35:1278–85.
- [107] Avenevoli S, Merikangas KR. Familial influences on adolescent smoking. *Addiction* 2003;98(Suppl 1):1–20.
- [108] Niu T, Chen C, Ni J, Wang B, Fang Z, Shao H, et al. Nicotine dependence and its familial aggregation in Chinese. *Int J Epidemiol* 2000;29:248–52.
- [109] Goode EL, Badzioch MD, Kim H, Gagnon F, Rozek LS, Edwards KL, et al. Multiple genome-wide analyses of smoking behavior in the Framingham Heart Study. *BMC Genet* 2003;4(Suppl 1):S102.
- [110] Bergen AW, Yang XR, Bai Y, Beerman MB, Goldstein AM, Goldin LR. Genomic regions linked to alcohol consumption in the Framingham Heart Study. *BMC Genet* 2003;4(Suppl 1):S101.
- [111] Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V, Nurnberger Jr JI, et al. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. *Arch Gen Psychiatry* 1998;55:982–8.
- [112] Osler M, Holst C, Prescott E, Sorensen TI. Influence of genes and family environment on adult smoking behavior assessed in an adoption study. *Genet Epidemiol* 2001;21:193–200.
- [113] Heath AC, Martin NG. Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. *Addict Behav* 1993;18:19–34.
- [114] Madden PA, Heath AC, Pedersen NL, Kaprio J, Koskenvuo MJ, Martin NG. The genetics of smoking persistence in men and women: a multicultural study. *Behav Genet* 1999;29:423–31.
- [115] Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA. A population-based twin study in women of smoking initiation and nicotine dependence. *Psychol Med* 1999;29:299–308.
- [116] Neale MC, Harvey E, Maes HH, Sullivan PF, Kendler KS. Extensions to the modeling of initiation and progression: applications to substance use and abuse. *Behav Genet* 2006;36:507–24.
- [117] Breslau N, Novak SP, Kessler RC. Psychiatric disorders and stages of smoking. *Biol Psychiatry* 2004;55:69–76.
- [118] Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA. Nicotine dependence and psychiatric disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. *Arch Gen Psychiatry* 2004;61:1107–15.
- [119] de Leon J, Becona E, Gurpegui M, Gonzalez-Pinto A, Diaz FJ. The association between high nicotine dependence and severe mental illness may be consistent across countries. *J Clin Psychiatry* 2002;63:812–6.
- [120] Hughes JR. Comorbidity and smoking. *Nicotine Tob Res* 1999;1(Suppl 2):S149–52. discussion S65–S66.
- [121] John U, Meyer C, Rumpf HJ, Hapke U. Smoking, nicotine dependence and psychiatric comorbidity—a population-based study including smoking cessation after three years. *Drug Alcohol Depend* 2004;76:287–95.
- [122] Keel PK, Klump KL, Miller KB, McGue M, Iacono WG. Shared transmission of eating disorders and anxiety disorders. *Int J Eat Disord* 2005;38:99–105.
- [123] Volk HE, Scherrer JF, Bucholz KK, Todorov A, Heath AC, Jacob T, et al. Evidence for specificity of transmission of alcohol and nicotine dependence in an offspring of twins design. *Drug Alcohol Depend* 2007;87:225–32.
- [124] Hopfer CJ, Stallings MC, Hewitt JK. Common genetic and environmental vulnerability for alcohol and tobacco use in a volunteer sample of older female twins. *J Stud Alcohol* 2001;62:717–23.
- [125] Koopmans JR, van Doornen LJ, Boomsma DI. Association between alcohol use and smoking in adolescent and young adult twins: a bivariate genetic analysis. *Alcohol Clin Exp Res* 1997;21:537–46.
- [126] Madden PA, Heath AC. Shared genetic vulnerability in alcohol and cigarette use and dependence. *Alcohol Clin Exp Res* 2002;26:1919–21.
- [127] Bierut LJ, Rice JP, Goate A, Hinrichs AL, Saccone NL, Foroud T, et al. A genomic scan for habitual smoking in families of alcoholics: common and specific genetic factors in substance dependence. *Am J Med Genet A* 2004;124:19–27.
- [128] Dick DM, Nurnberger Jr J, Edenberg HJ, Goate A, Crowe R, Rice J, et al. Suggestive linkage on chromosome 1 for a quantitative alcohol-related phenotype. *Alcohol Clin Exp Res* 2002;26:1453–60.
- [129] Peterson LE, Barnholtz JS, Page GP, King TM, de Andrade M, Amos CA. A genome-wide search for susceptibility genes linked to alcohol dependence. *Genet Epidemiol* 1999;17(Suppl 1):S295–300.
- [130] Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, et al. Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* 1998;81:207–15.
- [131] Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N, Edenberg HJ, et al. A genome screen of maximum number of drinks as an alcoholism phenotype. *Am J Med Genet* 2000;96:632–7.
- [132] Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T, et al. A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* 2001;25:323–9.
- [133] Ye Y, Zhong X, Zhang H. A genome-wide tree- and forest-based association analysis of comorbidity of alcoholism and smoking. *BMC Genet* 2005;6(Suppl 1):S135.
- [134] Ehringer MA, Clegg HV, Collins AC, Corley RP, Crowley T, Hewitt JK, et al. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNA2) with subjective responses to alcohol and nicotine. *Am J Med Genet B Neuropsychiatr Genet* 2007;144:596–604.
- [135] Dierker LC, Avenevoli S, Stolar M, Merikangas KR. Smoking and depression: an examination of mechanisms of comorbidity. *Am J Psychiatry* 2002;159:947–53.

- [136] Kendler KS, Neale MC, MacLean CJ, Heath AC, Eaves LJ, Kessler RC. Smoking and major depression. A causal analysis. *Arch Gen Psychiatry* 1993;50:36–43.
- [137] Kendler KS, Gardner CO. Monozygotic twins discordant for major depression: a preliminary exploration of the role of environmental experiences in the aetiology and course of illness. *Psychol Med* 2001;31:411–23.
- [138] McCaffery JM, Niaura R, Swan GE, Carmelli D. A study of depressive symptoms and smoking behavior in adult male twins from the NHLBI twin study. *Nicotine Tob Res* 2003;5:77–83.
- [139] Lyons MJ, Bar JL, Kremen WS, Toomey R, Eisen SA, Goldberg J, et al. Nicotine and familial vulnerability to schizophrenia: a discordant twin study. *J Abnorm Psychol* 2002;111:687–93.
- [140] Wang D, Ma JZ, Li MD. Mapping and verification of susceptibility loci for smoking quantity using permutation linkage analysis. *Pharmacogenom J* 2005;5:166–72.
- [141] Morley KI, Medland SE, Ferreira MA, Lynskey MT, Montgomery GW, Heath AC, et al. A possible smoking susceptibility locus on chromosome 11p12: evidence from sex-limitation linkage analyses in a sample of Australian twin families. *Behav Genet* 2006;36:87–99.
- [142] Gelernter J, Liu X, Hesselbrock V, Page GP, Goddard A, Zhang H. Results of a genomewide linkage scan: support for chromosomes 9 and 11 loci increasing risk for cigarette smoking. *Am J Med Genet B Neuropsychiatr Genet* 2004;128:94–101.
- [143] Li MD, Ma JZ, Cheng R, Dupont RT, Williams NJ, Crews KM, et al. A genome-wide scan to identify loci for smoking rate in the Framingham Heart Study population. *BMC Genet* 2003;4(Suppl 1):S103.
- [144] Saccone SF, Pergadia ML, Loukola A, Broms U, Montgomery GW, Wang JC, et al. Genetic linkage to chromosome 22q12 for a heavy-smoking quantitative trait in two independent samples. *Am J Hum Genet* 2007;80:856–66.
- [145] Risch N. Searching for genes in complex diseases: lessons from systemic lupus erythematosus. *J Clin Invest* 2000;105:1503–6.
- [146] Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, et al. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997;7:479–84.
- [147] Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 2004;23:540–5.
- [148] Yu Y, Panhuysen C, Kranzler HR, Hesselbrock V, Rounsaville B, Weiss R, et al. Intronic variants in the dopa decarboxylase (DDC) gene are associated with smoking behavior in European-Americans and African-Americans. *Hum Mol Genet* 2006;15:2192–9.
- [149] Ma JZ, Beuten J, Payne TJ, Dupont RT, Elston RC, Li MD. Haplotype analysis indicates an association between the DOPA decarboxylase (DDC) gene and nicotine dependence. *Hum Mol Genet* 2005;14:1691–8.
- [150] Beuten J, Payne TJ, Ma JZ, Li MD. Significant association of catechol-O-methyltransferase (COMT) haplotypes with nicotine dependence in male and female smokers of two ethnic populations. *Neuropsychopharmacology* 2006;31:675–84.
- [151] Beuten J, Ma JZ, Lou XY, Payne TJ, Li MD. Association analysis of the protein phosphatase 1 regulatory subunit 1B (PPP1R1B) gene with nicotine dependence in European- and African-American smokers. *Am J Med Genet B Neuropsychiatr Genet* 2007;144:285–90.
- [152] Li MD, Beuten J, Ma JZ, Payne TJ, Lou XY, Garcia V, et al. Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. *Hum Mol Genet* 2005;14:1211–9.
- [153] Lou XY, Ma JZ, Payne TJ, Beuten J, Crew KM, Li MD. Gene-based analysis suggests association of the nicotinic acetylcholine receptor beta1 subunit (CHRNA1) and M1 muscarinic acetylcholine receptor (CHRM1) with vulnerability for nicotine dependence. *Hum Genet* 2006;120:381–9.
- [154] Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, et al. A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. *Mol Psychiatry* 2002;7:224–8.
- [155] Fillingim RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, et al. The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. *J Pain* 2005;6:159–67.
- [156] Beuten J, Ma JZ, Payne TJ, Dupont RT, Crews KM, Somes G, et al. Single- and multilocus allelic variants within the GABA(B) receptor subunit 2 (GABAB2) gene are significantly associated with nicotine dependence. *Am J Hum Genet* 2005;76:859–64.
- [157] Beuten J, Ma JZ, Payne TJ, Dupont RT, Lou XY, Crews KM, et al. Association of specific haplotypes of neurotrophic tyrosine kinase receptor 2 gene (NTRK2) with vulnerability to nicotine dependence in African-Americans and European-Americans. *Biol Psychiatry* 2007;61:48–55.
- [158] Li MD, Sun D, Lou XY, Beuten J, Payne TJ, Ma JZ. Linkage and association studies in African- and Caucasian-American populations demonstrate that SHC3 is a novel susceptibility locus for nicotine dependence. *Mol Psychiatry* 2007;12:462–73.
- [159] Beuten J, Ma JZ, Payne TJ, Dupont RT, Quezada P, Huang W, et al. Significant association of BDNF haplotypes in European-American male smokers but not in European-American female or African-American smokers. *Am J Med Genet B Neuropsychiatr Genet* 2005;139:73–80.
- [160] Lindstrom JM. Nicotinic acetylcholine receptors of muscles and nerves: comparison of their structures, functional roles, and vulnerability to pathology. *Ann N Y Acad Sci* 2003;998:41–52.
- [161] Uhl GR, Liu QR, Drong T, Johnson C, Walther D, Rose JE. Molecular genetics of nicotine dependence and abstinence: whole genome association using 520,000 SNPs. *BMC Genet* 2007;8:10.
- [162] Cardon LR, Bell JL. Association study designs for complex diseases. *Nat Rev Genet* 2001;2:91–9.
- [163] McClure JB, Swan GE. Tailoring nicotine replacement therapy: rationale and potential approaches. *CNS Drugs* 2006;20:281–91.
- [164] A clinical practice guideline for treating tobacco use and dependence: a US Public Health Service report. The Tobacco Use and Dependence Clinical Practice Guideline Panel, Staff, and Consortium Representatives. *J Am Med Assoc* 2000;283:3244–54.
- [165] Arinami T, Gao M, Hamaguchi H, Toru M. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. *Hum Mol Genet* 1997;6:577–82.
- [166] Lerman C, Jepson C, Wileyto EP, Epstein LH, Rukstalis M, Patterson F, et al. Role of functional genetic variation in the dopamine D2 receptor (DRD2) in response to bupropion and nicotine replacement therapy for tobacco dependence: results of two randomized clinical trials. *Neuropsychopharmacology* 2006;31:231–42.



- [167] Dahl JP, Jepson C, Levenson R, Wileyto EP, Patterson F, Berrettini WH, et al. Interaction between variation in the D2 dopamine receptor (DRD2) and the neuronal calcium sensor-1 (FREQ) genes in predicting response to nicotine replacement therapy for tobacco dependence. *Pharmacogenom J* 2006;6:194–9.
- [168] Duan J, Wainwright MS, Comeran JM, Saitou N, Sanders AR, Gelernter J, et al. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet* 2003;12:205–16.
- [169] Johnstone EC, Yudkin PL, Hey K, Roberts SJ, Welch SJ, Murphy MF, et al. Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. *Pharmacogenetics* 2004;14:83–90.
- [170] Yudkin P, Munafò M, Hey K, Roberts S, Welch S, Johnstone E, et al. Effectiveness of nicotine patches in relation to genotype in women versus men: randomised controlled trial. *Br Med J* 2004;328:989–90.
- [171] Berlin I, Covey LS, Jiang H, Hamer D. Lack of effect of D2 dopamine receptor TaqI A polymorphism on smoking cessation. *Nicotine Tob Res* 2005;7:725–8.
- [172] Lerman C, Shields PG, Wileyto EP, Audrain J, Hawk Jr LH, Pinto A, et al. Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. *Health Psychol* 2003;22:541–8.
- [173] Swan GE, Jack LM, Valdes AM, Ring HZ, Ton CC, Curry SJ, et al. Joint effect of dopaminergic genes on likelihood of smoking following treatment with bupropion SR. *Health Psychol* 2007;26:361–8.
- [174] van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, et al. Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. *J Nucl Med* 2005;46:745–51.
- [175] O'Gara C, Stapleton J, Sutherland G, Guindalini C, Neale B, Breen G, et al. Dopamine transporter polymorphisms are associated with short-term response to smoking cessation treatment. *Pharmacogenet Genom* 2007;17:61–7.
- [176] Colilla S, Lerman C, Shields PG, Jepson C, Rukstalis M, Berlin J, et al. Association of catechol-O-methyltransferase with smoking cessation in two independent studies of women. *Pharmacogenet Genom* 2005;15:393–8.
- [177] Hughes JR, Stead LF, Lancaster T. Antidepressants for smoking cessation. *Cochrane Database Syst Rev* 2002;CD000031.
- [178] David SP, Munafò MR, Murphy MF, Walton RT, Johnstone EC. The serotonin transporter 5-HTTLPR polymorphism and treatment response to nicotine patch: follow-up of a randomized controlled trial. *Nicotine Tob Res* 2007;9:225–31.
- [179] Munafò MR, Johnstone EC, Wileyto EP, Shields PG, Elliot KM, Lerman C. Lack of association of 5-HTTLPR genotype with smoking cessation in a nicotine replacement therapy randomized trial. *Cancer Epidemiol Biomarkers Prev* 2006;15:398–400.
- [180] Johnstone E, Benowitz N, Cargill A, Jacob R, Hinks L, Day I, et al. Determinants of the rate of nicotine metabolism and effects on smoking behavior. *Clin Pharmacol Ther* 2006;80:319–30.
- [181] Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F, Tyndale RF. Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. *Mol Psychiatry* 2006;11:400–9.
- [182] Audrain-McGovern J, Al Koudsi N, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. *Pediatrics* 2007;119:e264–74.
- [183] Benowitz NL, Swan GE, Jacob 3rd P, Lessov-Schlaggar CN, Tyndale RF. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clin Pharmacol Ther* 2006;80:457–67.
- [184] Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, et al. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clin Pharmacol Ther* 2006;79:600–8.
- [185] Lee AM, Jepson C, Hoffmann E, Epstein L, Hawk LW, Lerman C, et al. CYP2B6 genotype alters abstinence rates in a bupropion smoking cessation trial. *Biol Psychiatry* 2007;62:635–41.
- [186] Nides M, Oncken C, Gonzales D, Rennard S, Watsky EJ, Anziano R, et al. Smoking cessation with varenicline, a selective alpha4beta2 nicotinic receptor partial agonist: results from a 7-week, randomized, placebo- and bupropion-controlled trial with 1-year follow-up. *Arch Intern Med* 2006;166:1561–8.
- [187] Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *J Am Med Assoc* 2006;296:47–55.
- [188] Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, et al. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *J Am Med Assoc* 2006;296:56–63.
- [189] Munafò MR, Johnstone EC. Effects of dopamine D2 receptor gene polymorphisms on smoking cessation: abstinence and withdrawal symptoms. *Pharmacogenomics* 2007;8:513–7.
- [190] Munafò M, Lerman C. Can pharmacogenetics help smokers quit? *Pharmacogenomics* 2006;7:1137–40.
- [191] Shields A, Lerman C, Sullivan P. Translating emerging research on the genetics of smoking into clinical practice: ethical and social considerations. *Nicotine Tob Res* 2004;6:675–88.
- [192] Berrettini WH, Lerman CE. Pharmacotherapy and pharmacogenetics of nicotine dependence. *Am J Psychiatry* 2005;162:1441–51.
- [193] Hall WD. A research agenda for assessing the potential contribution of genomic medicine to tobacco control. *Tob Control* 2007;16:53–8.
- [194] Dick DM, Viken R, Purcell S, Kaprio J, Pulkkinen L, Rose RJ. Parental monitoring moderates the importance of genetic and environmental influences on adolescent smoking. *J Abnorm Psychol* 2007;116:213–8.
- [195] Timberlake DS, Rhee SH, Haberstick BC, Hopfer C, Ehringer M, Lessem JM, et al. The moderating effects of religiosity on the genetic and environmental determinants of smoking initiation. *Nicotine Tob Res* 2006;8:123–33.
- [196] Dick DM, Rose RJ, Viken RJ, Kaprio J, Koskenvuo M. Exploring gene-environment interactions: socioregional moderation of alcohol use. *J Abnorm Psychol* 2001;110:625–32.
- [197] Heath AC, Jardine R, Martin NG. Interactive effects of genotype and social environment on alcohol consumption in female twins. *J Stud Alcohol* 1989;50:38–48.
- [198] Koopmans JR, Slutske WS, van Baal GC, Boomsma DI. The influence of religion on alcohol use initiation: evidence for genotype X environment interaction. *Behav Genet* 1999;29:445–53.
- [199] Miles DR, Silberg JL, Pickens RW, Eaves LJ. Familial influences on alcohol use in adolescent female twins:

- testing for genetic and environmental interactions. *J Stud Alcohol* 2005;66:445–51.
- [200] Rose RJ, Dick DM, Viken And RJ, Kaprio J. Gene-environment interaction in patterns of adolescent drinking: regional residency moderates longitudinal influences on alcohol use. *Alcohol Clin Exp Res* 2001;25:637–43.
- [201] Heath AC, Eaves LJ, Martin NG. Interaction of marital status and genetic risk for symptoms of depression. *Twin Res* 1998;1:119–22.
- [202] Boomsma DI, de Geus EJ, van Baal GC, Koopmans JR. A religious upbringing reduces the influence of genetic factors on disinhibition: evidence for interaction between genotype and environment on personality. *Twin Res* 1999;2:115–25.
- [203] Tuvblad C, Grann M, Lichtenstein P. Heritability for adolescent antisocial behavior differs with socioeconomic status: gene–environment interaction. *J Child Psychol Psychiatry* 2006;47:734–43.
- [204] Harden KP, Turkheimer E, Loehlin JC. Genotype by environment interaction in adolescents' cognitive aptitude. *Behav Genet* 2007;37:273–83.
- [205] Turkheimer E, Haley A, Waldron M, D'Onofrio B, Gottesman II. Socioeconomic status modifies heritability of IQ in young children. *Psychol Sci* 2003;14:623–8.
- [206] Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003;301:386–9.
- [207] Dick DM, Plunkett J, Hamlin D, Nurnberger Jr J, Kuperman S, Schuckit M, et al. Association analyses of the serotonin transporter gene with lifetime depression and alcohol dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) sample. *Psychiatr Genet* 2007;17:35–8.
- [208] Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry* 2005;62:529–35.
- [209] Taylor SE, Way BM, Welch WT, Hilmert CJ, Lehman BJ, Eisenberger NI. Early family environment, current adversity, the serotonin transporter promoter polymorphism, and depressive symptomatology. *Biol Psychiatry* 2006;60:671–6.
- [210] Chipman P, Jorm AF, Prior M, Sanson A, Smart D, Tan X, et al. No interaction between the serotonin transporter polymorphism (5-HTTLPR) and childhood adversity or recent stressful life events on symptoms of depression: results from two community surveys. *Am J Med Genet B Neuropsychiatr Genet* 2007;144:561–5.
- [211] Gillespie NA, Whitfield JB, Williams B, Heath AC, Martin NG. The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression. *Psychol Med* 2005;35:101–11.
- [212] Chorbv VM, Lobos EA, Todorov AA, Heath AC, Botteron KN, Todd RD. Relationship of 5-HTTLPR genotypes and depression risk in the presence of trauma in a female twin sample. *Am J Med Genet B Neuropsychiatr Genet* 2007.
- [213] Lerer E, Kanyas K, Karni O, Ebstein RP, Lerer B. Why do young women smoke? II. Role of traumatic life experience, psychological characteristics and serotonergic genes. *Mol Psychiatry* 2006;11:771–81.
- [214] Schmitt JE, Prescott CA, Gardner CO, Neale MC, Kendler KS. The differential heritability of regular tobacco use based on method of administration. *Twin Res Hum Genet* 2005;8:60–2.
- [215] Carmelli D, Swan GE, Robinette D, Fabsitz R. Genetic influence on smoking—a study of male twins. *N Engl J Med* 1992;327:829–33.
- [216] Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tob Control* 2003;12:424–30.
- [217] Fowler JS, Logan J, Wang GJ, Volkow ND. Monoamine oxidase and cigarette smoking. *Neurotoxicology* 2003;24:75–82.
- [218] Berlin I, Said S, Spreux-Varoquaux O, Olivares R, Launay JM, Puech AJ. Monoamine oxidase A and B activities in heavy smokers. *Biol Psychiatry* 1995;38:756–61.
- [219] Castagnoli K, Steyn SJ, Magnin G, Van Der Schyf CJ, Fourie I, Khalil A, et al. Studies on the interactions of tobacco leaf and tobacco smoke constituents and monoamine oxidase. *Neurotox Res* 2002;4:151–60.
- [220] Berlin I, Anthenelli RM. Monoamine oxidases and tobacco smoking. *Int J Neuropsychopharmacol* 2001;4:33–42.
- [221] Orelund L, Fowler CJ, Schalling D. Low platelet monoamine oxidase activity in cigarette smokers. *Life Sci* 1981;29:2511–8.
- [222] Pavlin R, Sket D. Effect of cigarette smoke on brain monoamine oxidase activity. *Farmacvtski Vestnik* 1983;44:185–92.
- [223] Villégier AS, Blanc G, Glowinski J, Tassin JP. Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. *Pharmacol Biochem Behav* 2003;76:267–74.
- [224] Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res* 1993;18:247–91.
- [225] Villégier AS, Lotfipour S, McQuown SC, Belluzzi JD, Leslie FM. Tranlycypromine enhancement of nicotine self-administration. *Neuropharmacology* 2007;52:1415–25.
- [226] Villégier AS, Salomon L, Granon S, Changeux JP, Belluzzi JD, Leslie FM, et al. Monoamine oxidase inhibitors allow locomotor and rewarding responses to nicotine. *Neuropsychopharmacology* 2006;31:1704–13.
- [227] Seeman JJ, Dixon M, Haussmann HJ. Acetaldehyde in mainstream tobacco smoke: formation and occurrence in smoke and bioavailability in the smoker. *Chem Res Toxicol* 2002;15:1331–50.
- [228] Talhout R, Opperhuizen A, van Amsterdam JG. Role of acetaldehyde in tobacco smoke addiction. *Eur Neuropsychopharmacol* 2007;17:627–36.
- [229] Belluzzi JD, Wang R, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. *Neuropsychopharmacology* 2005;30:705–12.
- [230] Cao J, Belluzzi JD, Loughlin SE, Keyler DE, Pentel PR, Leslie FM. Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. *Neuropsychopharmacology* 2007;32:2025–35.
- [231] Swan GE, Lessov CN. Gene-environment interaction in nicotine addiction: the need for a large-scale, collaborative effort. *Subst Use Misuse* 2004;39:2083–5.
- [232] Swan GE. Implications of genetic epidemiology for the prevention of tobacco use. *Nicotine Tob Res* 1999;1(Suppl 1):S49–56.
- [233] Swan GE. The need for dissemination of evidence-based results from research on nicotine and tobacco. *Nicotine Tob Res* 2003;5:7–8.
- [234] Bierut LJ, Cubells JF, Iacono WG, Li MD, Madden PA, Nelson EC, et al. Genetic research and smoking behavior. *J Am Med Assoc* 2007;297:809 [author reply 10].
- [235] Raaschou-Nielsen E. Smoking habits in twins. *Dan Med Bull* 1960;7:82–8.