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Status of Genetic Studies of Nicotine Dependence

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This chapter frames important issues in identifying potential phenotypes of nicotine dependence and sets the stage for examining the role of genetics in nicotine-dependence research. Key areas discussed include

- *Issues in the definition and measurement of nicotine dependence*
- *A framework for phenotypes for nicotine dependence that potentially links genetics and behavioral traits while showing measurable validity, reliability, and heritability*
- *The implications of epidemiological concepts in identifying potentially complex genetic risk factors for nicotine dependence*
- *Measuring environmental influences and including them in models of estimates of genetic risk and the role epigenetic investigations will play in future investigations*
- *A review of selected biometric and genetic studies of nicotine dependence*
- *The communication and interpretation of findings from genetic studies of nicotine dependence, including the need for replication, the potential for stigmatization, and value of direct-to-consumer marketing of genetic tests based on these findings*

This volume examines conceptual, theoretical, and methodological considerations in the development of nicotine-dependence phenotypes and endophenotypes. Each of these areas shows the potential for future study to help better understand factors in global tobacco use.

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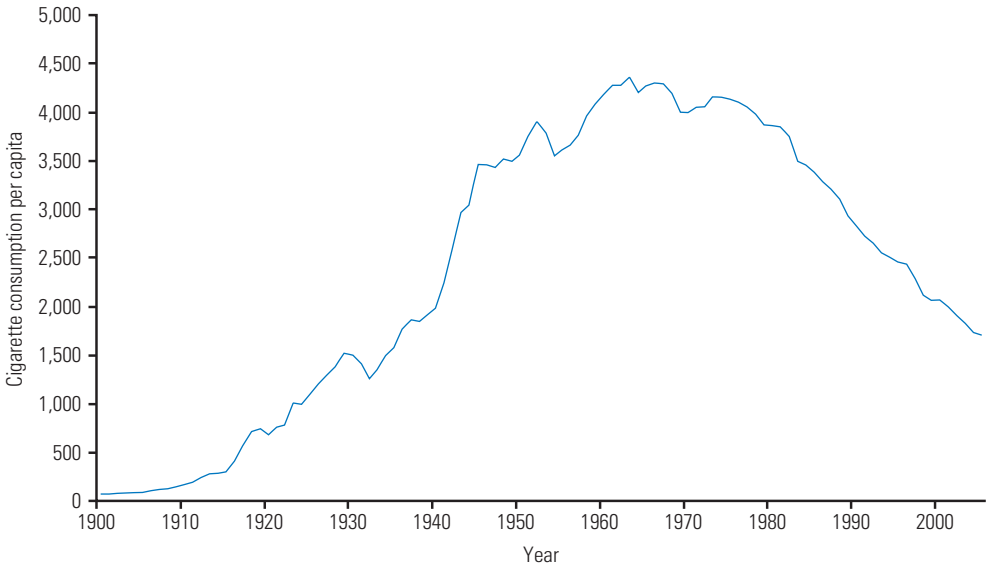
Introduction

Environmental influences on tobacco use in the United States have intensified dramatically over time and account for the sharp reduction in the overall prevalence of smoking (figure 2.1). An enormous body of literature, evolving separately from that on genetics and nicotine dependence, clearly documents the effect of specific environmental influences on the likelihood of exposure to tobacco, its regular use, its chronic use, and the difficulty some people have in stopping use. Protobacco stimuli are ubiquitous in the environment and include advertising by the tobacco industry and the portrayal of smoking in movies. Equally important, the tobacco industry controls the design of cigarettes and, so, the bioavailabilty of nicotine. The form in which the nicotine is delivered is an important variable that almost certainly interacts with the biological factors discussed in this report. Similarly, antitobacco stimuli have become almost as widespread in some parts of the world. Antismoking media, smoke-free workplaces and public places, smoke-free homes, concern over secondhand smoke, and the pricing of tobacco products are a few of the sources of environmental variation.

The marked decline in cigarette consumption in the United States since the 1960s (most of which has taken place since 1981 [640 billion cigarettes consumed compared to an estimate of 371 billion cigarettes in 2006]) corresponds to increased public awareness of the dangers of tobacco use, changing social norms about tobacco, and increased governmental actions to regulate the use, sale, and advertising of tobacco products. The most comprehensive environmental changes have been in attitudes and rules about smoking in enclosed public places. As late as 20 years ago, smoking was ubiquitous in most places, with smoking allowed virtually everywhere (unless it posed a danger of fires or damage

Figure 2.1 Cigarette Consumption in the United States in the Twentieth Century



Note. From National Center for Health Statistics, Centers for Disease Control and Prevention, 2002. Cigarette consumption: U.S. Department of Agriculture, 1900–2000.

to equipment). Over time, the environment that had supported smoking indoors has transformed. Limiting where people can smoke has contributed to the social marginalization of smoking as an accepted behavior. In addition, another major reason for this decline is the associated rise in price per pack from about $1.50 in 1980 to more than $4.20 in 2007.[1]

However, despite price increases and intensive public health control, an estimated 45 million people in the United States still smoke (17 million attempt to quit annually),[2] testifying to the fact that the consistent application of already effective methods of prevention and intervention is necessary to further reduce the prevalence of tobacco use in this country.[3] The annual cost to the U.S. economy is estimated to be $167 billion due to premature death and disability.[2]

It is estimated that approximately 1 billion people worldwide are regular users of tobacco (96.3% of smokers are outside of the United States) and that 3–6 million people die every year from tobacco-related illnesses.[4] This number is expected to climb to 9 million by the year 2030.[5] The prevalence of smoking outside the United States varies widely but is as high as 60% among men in some countries. The prevalence of smoking among non-American women is generally lower but appears to be rising in some countries as "westernization" continues.[6] These data suggest that the effects of culture are another important aspect of environmental influences. For example, in many cultures, very few women smoke. The fact that more women start to smoke when moving from these cultures to the United States (or other places where smoking by women is accepted), or when exposed to cigarette marketing targeted to women, demonstrates dramatically the power of the environment to influence nicotine dependence. On a population-wide basis, the great diversity in tobacco use behaviors observed both between countries and within countries over time demonstrates that biology alone cannot fully explain variations in tobacco use behaviors. These statistics indicate that the demand for both prevention and intervention efforts at tobacco control will continue to increase and will become urgent as the costs to existing and emerging economies are realized. All available tools will be needed to meet the demand for effective and sustainable tobacco control, including pharmacogenetic-informed treatments and social policy interventions[7] for smoking cessation.

The highly addictive nature of nicotine and the more than $13 billion spent annually by the tobacco industry[8] to market its products to the American people contribute much to influence new and continuing smokers. However, the majority of adults and children choose not to use tobacco products. The answer to the question of intense scientific interest, "Why do some people smoke and others do not?" remains as elusive today as it was in 1993 when it was articulated by Pomerleau and colleagues.[9]

Although work in the human domain as well as in animal models has contributed to knowledge of the processes and pathways underlying nicotine dependence specifically, and addiction more generally, it is fair to say that knowledge derived from genetic studies of nicotine dependence has yet to inform prevention or cessation efforts. This has led some to conclude that research on nicotine dependence should be given a lower priority in the search for genes for complex disorders.[10,11] However, given the large public health burden of tobacco use, the continued influx of new tobacco users, and the demands of sustained smoking cessation, it is imperative that the search for answers continues unabated.[12,13] Environmental modification for the prevention and management of common conditions has been beneficial, but generic interventions should be supplemented by specifically targeted treatment based on a more precise

knowledge of biological mechanisms if further progress is to be made.[14]

Papers such as those by Merikangas and Risch[10] and Carlsten and Burke[11] do not address the fact that complex traits such as nicotine dependence are multiply determined and treated. Previously, an integrative model of tobacco use and nicotine dependence was described[15] (figure 2.2) that recognizes the role played by individual differences in vulnerability factors,[16] in tobacco use trajectories,[17–20] in environmental exposure,[21] and in nicotine metabolism and nicotine dependence, including motivations to smoke and the reinforcement derived from tobacco.[22,23] Certain factors such as anxiety, depression, use of other substances, and family history of tobacco use, along with individual differences in nicotine sensitivity or metabolism, might themselves have genetic components.[16] It has been suggested that the effects of these variables on subsequent likelihood of smoking are mediated by personal factors such as lower performance on certain tests of cognition, socioeconomic status, and the occurrence of events within the social environment such as having peers who smoke and family discord.[16,24]

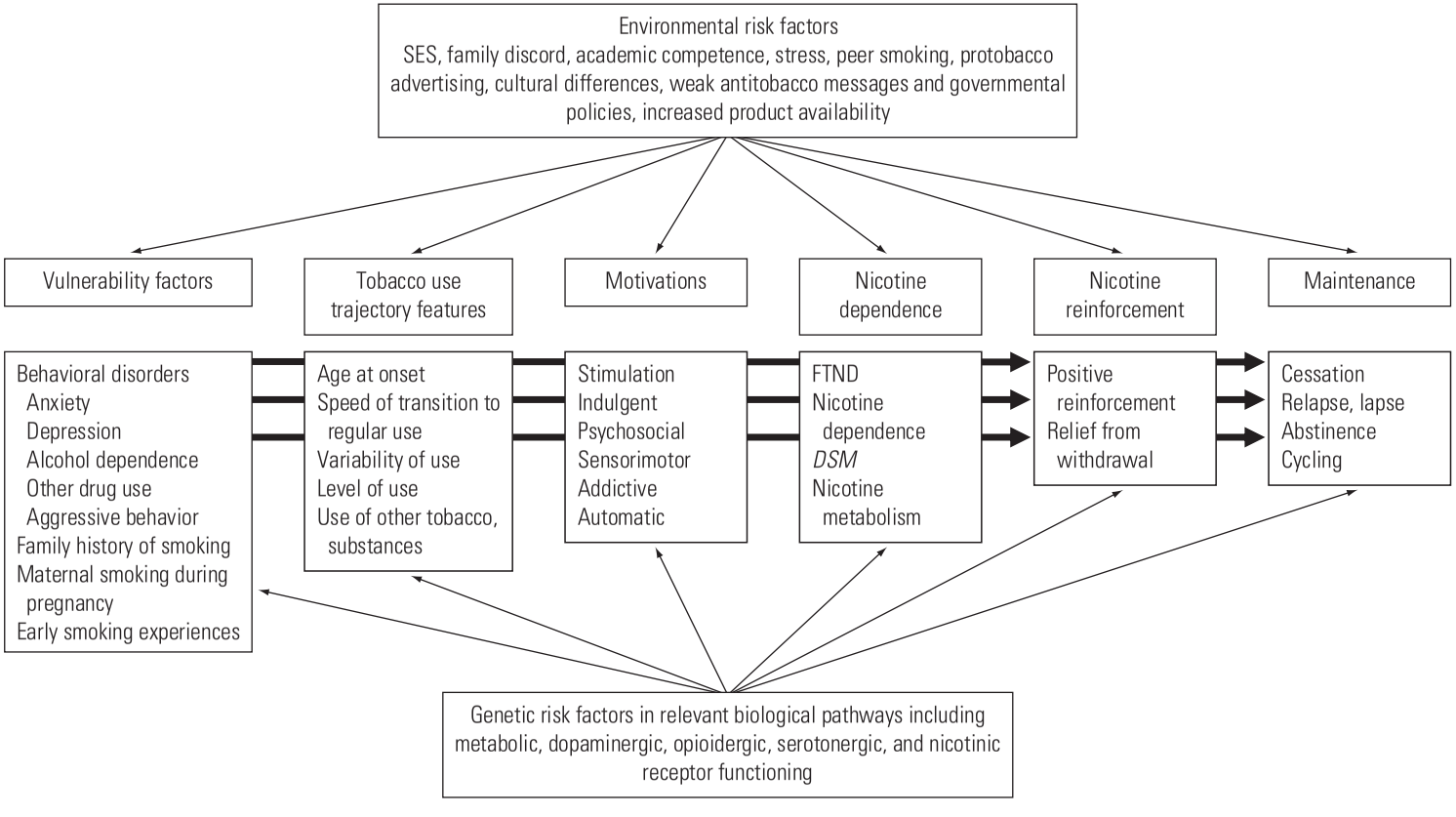
Nicotine, the psychoactive alkaloid found in tobacco products, is thought to play a major role in nicotine dependence. Most smokers tend to ingest similar amounts of nicotine from day to day, consistent with the idea that they titrate their dose of nicotine to achieve desired effects.[25] Nicotine is extensively metabolized in the body, primarily by the liver cytochrome P-450 enzyme CYP2A6.[26,27] Some studies have shown that the rate of nicotine metabolism may be related to nicotine-dependence risk.[28,29] Because CYP2A6 activity affects the rate at which nicotine is eliminated, genetic alterations in the CYP2A6 enzyme may affect smoking behavior and nicotine dependence, and this deserves additional attention. Other genetic factors that may contribute to nicotine dependence include variation in pathways responsible for nicotine reward and pleasure.[30–32] An important feature of the model for tobacco use over the life span is that genetic and environmental factors exerting influence at different points in the development of tobacco use (e.g., initiation, maintenance, cessation, and relapse) are likely to be different.[33]

Another feature in figure 2.2 that requires further investigation by genetic studies of nicotine dependence concerns the plethora of environmental conditions that are recognized to play a role in the acquisition, maintenance, cessation, and relapse of smoking.[34] Simply put, with one exception in 2007,[35] genetic investigations of smoking did not incorporate environmental measures into their study designs. It has been shown that genetic risk for smoking is lower at higher levels of parental monitoring,[35] suggesting that parental monitoring may help counteract genetic susceptibility to smoking behavior.

Historical Perspective of Genetic Research on Nicotine Dependence

A focused research agenda on the genetic basis of nicotine dependence is a relatively new development, but there have been earlier studies and claims about a potential genetic or constitutional basis for smoking behavior. Beginning in the 1950s, surveys showed that smokers and nonsmokers differ on a number of characteristics, including personality, occupation, diet, and physical characteristics.[36,37] In addition, legendary British statistician Ronald Aylmer Fisher argued that a common cause, likely genetic or constitutional, might be responsible for a tendency to smoke and increased susceptibility to cancer. In two letters to *Nature,* Fisher described two small studies of twins suggesting that monozygotic twins,

Figure 2.2 Trajectories as Phenotypic Pathways



Note. SES = socioeconomic status; FTND = Fagerström Test for Nicotine Dependence; *DSM = Diagnostic and Statistical Manual of Mental Disorders*. Adapted from Swan, G. E., K. S. Hudmon, L. M. Jack, K. Hemberger, D. Carmelli, T. V. Khroyan, H. Z. Ring, et al. 2003. Environmental and genetic determinants of tobacco use: Methodology for a multidisciplinary, longitudinal family-based investigation. *Cancer Epidemiology, Biomarkers & Prevention* 12 (10): 994–1005.

even when separated at birth, tend to have similar smoking habits.[38] While the evidence was limited, Fisher argued that his primary purpose was to draw attention to the inadequacies of the epidemiological studies on smoking and health.[39] The landmark 1964 Surgeon General's report on smoking and health reviewed the evidence linking smoking behavior with various characteristics and concluded that there was "overwhelming" evidence that smoking was psychologically and socially determined but that there was not yet any consistent evidence for constitutional or hereditary factors.[40(p377)] Nevertheless, Fisher's proposal, which came to be known as the "constitutional hypothesis," was one of the central arguments used by the tobacco industry to question the emerging evidence on cigarettes.[41]

Although it was widely recognized that many smokers exhibited characteristics of dependence, this phenomenon was initially viewed as primarily psychological and social, rather than pharmacological.[42] The 1964 Surgeon General's report specifically concluded that tobacco dependence should be described as "habituation" rather than "addiction" to differentiate it from the effects of narcotics and other "more potent" addicting drugs.[40(p350)] During the early 1970s, a few pioneering scientists, notably Murray Jarvik and M.A.H. Russell, began studying smoking behavior and the role of nicotine to understand the dependence process.[43,44] Yet, it was not until the late 1970s, that a substantial contingent of behavioral scientists who had been studying other forms of drug addiction began to develop a research agenda around smoking;[45] and the 1979 Surgeon General's report was the first to devote substantial attention to smoking behavior and dependence.[46]

Research in humans involving the relationship between measured genetic factors and smoking was first reported in 1993.[47,48] Since the initial reports, many published papers have reported associations between smoking behaviors and variants in a number of candidate genes. In all cases, the effect sizes reported were modest in nature, and until 2007, the studies were small and relied upon broad categories of smoking behaviors. The studies also reported single gene associations, some of which included variants with no known functional consequence. At least four separate meta-analyses of the literature concluded, that after interstudy heterogeneity has been taken into account, the association between genes and smoking behavior is modest indeed.[49–52]

Approximately 25 linkage studies in families have reported cosegregation of smoking behaviors with specific genomic regions. Few to none of these reported linkages have been strong enough to be called significant by current standards, and interestingly, many of the genomic regions that have been identified do not contain candidate genes of interest. Before 1978, most of the studies relied upon study samples that were constructed for reasons other than the study of smoking, used broad or imprecise classification of smoking behaviors, and relied on relatively loosely spaced marker sets with intermarker distances of five centimorgans (cM) or more. The first attempt to map susceptibility loci for nicotine dependence per se used the Fagerström Tolerance Questionnaire (FTQ)[53] (nicotine dependence defined as a score of seven or more) in a convenience sample of 130 families from Christchurch, New Zealand;[54] the FTQ was the precursor of the Fagerström Test for Nicotine Dependence (FTND). While initial results by Straub and colleagues showed limited evidence for linkage with specific regions (the strongest being a sharp peak at or near D2S1326), a subsequent reanalysis of the same data with different methods detected the same peak with an estimated Z-score of about 2.5.[55]

Despite the cumulative results from work beginning in the early 1990s, there is still

no example in which these findings have made a difference to the early detection, prevention, or treatment of nicotine dependence. Collectively, the work in humans and animals has provided new insight into the underlying neurobiology by underlining the extreme complexity of nicotine dependence. In this regard, conclusions from the body of evidence from the first generation of measured genetic studies of nicotine dependence parallels similar conclusions from first-generation studies of other complex traits in general and those from psychiatric genetics more specifically.

Noting the problems of nonreplication in psychiatric genetics research, Caspi and Moffitt in 2006[56] identified three general approaches that have been taken in the literature. The first approach assumes direct linear relations between gene and behaviors, and this would be an accurate characterization of the bulk of the work on nicotine genetics summarized above. The second approach involves the use of intermediate phenotypes, also known as endophenotypes, that are related to an illness, are heritable, and could be neuropsychological, neurophysiological, biochemical, endocrinological, or neuroanatomical in nature. One of the assumptions of this approach is that these constituents will have simpler genetic underpinnings than does the disorder itself. The third approach involves the study of gene-environment interactions in which it is assumed that "environmental pathogens" cause a disorder such as nicotine dependence only in the presence of certain gene variants. The second and third approaches have yet to be fully exploited in the context of nicotine dependence. One of the objectives of the present monograph is to more fully explore the existing options to inform the next generation of genetic studies for all three of the research traditions for complex genetic traits.

The introduction of powerful, new genomic technologies will make previous research quickly obsolete. With the decrease in costs and the use of platforms to genotype individuals for very large numbers of variants across the whole genome, the genome-wide association study (GWAS) has now become possible. Similarly, it is now possible to genotype candidate genes not just for one variant but for many variants (known as single nucleotide polymorphisms [SNPs]), many of which are functional in nature by virtue of either their location or experimental validation. The first published example of this approach to the study of nicotine dependence and genes is summarized later in the section, "Genome-wide Association and Candidate Gene Studies of FTND."

Unfortunately, the definition and measurement of nicotine dependence has not kept pace with the increased precision in the genomic arena. There is vigorous debate over what constitutes the critical constituents of nicotine dependence, and a definition that most or all investigators can agree upon remains elusive. One of the assumptions of this present volume is that until progress is made in understanding and resolving the conceptual and measurement issues in nicotine dependence, the yield from the advances in genomic science to better understand nicotine dependence will not be fully realized.

Nicotine Dependence: A Construct in Need of Refinement

One of the most troubling aspects of the state of nicotine-dependence measurement is the oft-cited finding from Moolchan and colleagues[57] in which poor agreement between the two gold-standard measures of nicotine dependence, the FTND and the *Diagnostic and Statistical Manual of*

Mental Disorders (DSM), was documented. This paper found that the kappa estimate of concordance was only .2, not much better than that expected by chance alone. This initial finding was confirmed in adolescents.[58] Both papers agree that the two measures, although claiming to be assessing nicotine dependence, are, in fact, assessing two different groups of smokers. The *DSM*-based approach appears to place a heavier emphasis on psychiatric symptoms, while the FTND appears to place a heavier emphasis on physical symptoms.

A consensus has emerged in which nicotine dependence is viewed as multidimensional and, therefore, should be assessed and quantified accordingly.[9,23,57,59,60] Although it was pointed out earlier[59,61] that dependence has several dimensions, including physical, behavioral, and psychological components, the assessment of nicotine dependence has relied largely upon the FTQ[53] and the FTND[62] or *DSM* Fourth Edition *(DSM-IV)* criteria, an approach deriving from the need to include nicotine dependence in psychiatric nomenclature and classification and that attempts to adhere to classic definitions of drug dependence.[63,64] Although both paper-and-pencil and psychiatric diagnostic approaches have provided reliable definitions for use in many different types of studies, neither of the existing assessments relies upon test development approaches well grounded in psychometric theory.

Multidimensional scales for assessing nicotine dependence have been published. However, their incorporation into genetic studies (biometric or measured) is only just beginning and the question of *which* components of nicotine dependence have the most or least genetic influence has only been addressed since 2004. A study by Lessov and colleagues,[65] described later in the section "Heritability of Components of Nicotine Dependence in Adults," was the first to document the relative proportion of genetic and environmental influences on diagnostic nicotine-dependence criteria, thereby opening the way for future studies to investigate dependence at a more precise level. Swan and colleagues[66] (also summarized later in the section "Linkage Analysis of FTND and Other Indices of Nicotine Dependence") was the first linkage study to recognize the complexity of the nicotine-dependence phenotype by including multiple phenotypic markers in the analyses.

The literature on the test-retest reliability of self-reported tobacco use reveals that over short and longer intervals, reliability is substantial for summary measures of nicotine dependence. The FTQ and derivatives (mFTQ, FTND) have acceptable levels of test-retest reliability that range from .72 to .92 over intervals up to 1.8 years in length.[67–74] Alternative measures of nicotine dependence have comparable 2- to 10-week test-retest reliabilities.[22,72,75–84] Only one study has reported test-retest reliability over an interval consistent with that in typical population surveys (up to 12 years)[85] and found acceptable reliability for total FTQ (.62) and FTND (.72) scores.

A number of authors suggest that milestones in the development of smoking behavior and/or individual items from several of the nicotine-dependence measures may be good candidates for inclusion in a genetic study of nicotine dependence. For example, there is significant additive genetic variance for age first smoked[86,87] and individual items from the FTND as well as diagnostic nicotine-dependence criteria.[65,88,89] However, at the level of individual items, it is evident that more needs to be known about test-retest reliability over intervals consistent with those in population-based surveys. Reliability estimates are more variable (0–.90) for individual items from the FTQ,[67–69] the FTND,[68,85] and the Hooked on Nicotine Checklist.[80,82] Perhaps not surprisingly, reliability of recall for specific

smoking behaviors such as smoking status and cigarettes smoked per day (CPD) are highly reliable for intervals of 3 years[81,90,91] and up to 15 years.[85]

Initial reactions to the first experience with smoking have also been suggested as an interesting and potentially informative phenotype for further genetic investigations.[92] Initial sensitivity, perhaps related to genetic variation in metabolic, neural, and/or airway pathways, in combination with the social environment may well influence which adolescents who experiment with tobacco go on to become regular smokers. Initial reactions and tobacco use milestones could be easily assessed in prospective, longitudinal studies of adolescents during and after experimentation. More commonly, however, there is a need in large, population-based studies to assess these characteristics retrospectively in adults with tobacco use history. Initial findings suggest that reported age at first cigarette may be easier to recall than one's subjective reaction to the first cigarette ever tried.[93,94] The extent to which the circumstances surrounding tobacco use (e.g., stress levels, other smokers, and depression) can be recalled reliably is of major interest, given the previously noted need to test for the presence of gene-environment interactions. Chapter 3 addresses many of the most important issues surrounding the measurement of nicotine dependence.

Nicotine-Dependence Phenotypes: A Framework

In most previous behavioral genetic and genetic epidemiological studies, "smoking" has been assessed as a static phenotype—that is, as if the behavior is a trait that remains constant over time. However, a variety of studies from the developmental, epidemiological, psychiatric, and smoking literature suggests that smoking, in general, and the consumption of nicotine on a regular basis, specifically, is tremendously more complex than the simple trait perspective.[95] Not only do reasons and motivations for smoking vary across individuals, it is likely that motivations (biological, social, and psychological, individually and in combination with each other) vary within an individual across time and situations.[34,96,97]

The field of psychiatric genetics is an area fraught with numerous examples of nonreplication.[98] However, some investigators believe that endophenotypes,[99,100] relying on actual measurements of behavior, physiological responses, or biological characteristics, such as brain structure from imaging studies, will provide more replicable associations with genetic variants than have more general diagnostic measures.[101–104]

Endophenotypes are viewed as quantifiable components in the genes-to-behavior pathway and can be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological in nature. To be viewed as a viable candidate for use in a genetic study of nicotine dependence, an endophenotype must be (1) associated with nicotine dependence in the population, (2) heritable, (3) state independent, (4) cosegregated with nicotine dependence in families, and (5) present at a higher rate among unaffected relatives of those with nicotine dependence than in the general population. Waldman[105] further suggests that candidate endophenotypes should have good psychometric properties, such as test-retest reliability, and be normally distributed. On the basis of work in schizophrenia as an example, endophenotypes that meet all or most of the criteria listed above include prepulse inhibition (a measure of sensory motor gating deficits), eye-tracking

dysfunction, and working memory.[104,106] Other branches of psychiatry have adopted the endophenotypic approach to investigate bipolar disorder, depression, Alzheimer's disease, attention deficit hyperactivity disorder (ADHD), autism, alcoholism, and personality disorders. Work in the field of ADHD, in particular, has benefited from this approach.[107] Some concerns have been raised, however, that the endophenotype approach may lead researchers to conduct smaller, underpowered studies because it is assumed that the more proximal measures will result in a stronger genetic signal. In the field of nicotine dependence, this remains an empirical question.[108]

In the field of tobacco use, numerous possibilities exist in which the relationships of phenotypes to genetic factors may actually be larger should the full range of phenotypes be explored. A framework was developed to organize phenotype selection for genetic investigations of tobacco use largely on the extent to which they could provide progressively more-fine-grained markers of nicotine dependence.[15]

At the least specific level, categories of smoking status and measures of amount smoked (class I) are included. The bulk of the work on genetics and smoking has relied upon these relatively nonspecific measures. Chapter 10 presents examples of how the definition of even broad phenotypes can be improved to be more specific within the context of epidemiological research. At the next level (class II), specific measures of nicotine dependence and their constituents are included because, while these may be related to quantity smoked, they appear to measure additional dimensions of nicotine dependence not assessed by simple measures of quantity consumed. Along with nicotine dependence, also included are withdrawal symptoms, motivations to smoke, as well as smoking topography, and more-fine-grained measures of *how* and *why* people consume tobacco.

Underlying the class III designation is the assumption that *how* individuals *attain* regular tobacco use (e.g., a trajectory) may be just as important as the fact that they are or have been regular users of tobacco. At this level of specificity, time-based aspects of an individual's history with tobacco become important, including the rate, level, and variability at which adolescents progress to regular tobacco use. The authors of chapters 5, 6, and 7 take a deeper look at approaches to identify tobacco use trajectory subgroups, the feasibility of their use as phenotypes in genetic studies, and the extent to which conjoint trajectories (tobacco and alcohol use) appear to have heritable components. At the highest level of specificity—class IV in this scheme—putative biological or physiological markers of nicotine dependence are included, such as pharmacokinetics and pharmacodynamics of nicotine, changes in neuropsychological function in response to nicotine, and changes at the receptor level (function, density). Chapter 4 addresses the issue of neurobiological phenotypes in animal models that can be viewed as analogs to class IV phenotypes in the human condition.

Available evidence indicates that the phenotypic options also vary as to whether they can be measured reliably, have validity as constituents of nicotine dependence, and are heritable—all three characteristics being defining criteria of endophenotypes. The most consistent evidence available is for the more general class I phenotypes. The crude measures of smoking status and quantity smoked can be measured with reliability over limited time intervals and are consistently correlated with components of measures of nicotine dependence. Their heritability has been well documented in twin and family studies.

Heritability estimates vary depending on how a phenotype is defined and the types of tobacco users (never, occasional, regular) included in the phenotypic definition.

Twin studies have addressed the genetic and environmental contributions to variation in several class I tobacco use phenotypes: initiation of tobacco use, measures of cigarette-smoking patterns (such as regular or current smoking), and measures of quantity of use (such as number of CPD). Across studies, measures of quantity of cigarettes smoked have been shown to be significantly heritable with estimates of heritability ranging from 45% to 86% for number of CPD[65,87,109–113] and 46% to 49% for heavy smoking.[114,115]

Lifetime regular smoking (a class I phenotype) often has been defined as having smoked 100 or more cigarettes in a lifetime and having exhibited a regular pattern of cigarette smoking. Regular smoking has been repeatedly shown to be moderately to highly heritable with an average estimate of approximately 50%.[102,116–119] Smoking persistence (also a class I phenotype), defined as being a current smoker versus a past smoker, also has been consistently shown to be heritable, with genetic influences estimated at 27% to 82%.[33,117,120–123]

Smoking initiation (class I) has generally been defined as a "yes" response to a question assessing whether a respondent has ever smoked or has ever tried smoking, but some studies have used an operationalization of smoking initiation (having smoked 100+ cigarettes) that could more accurately be described as ever smoking.[98,117,120,121,123] The smoking initiation phenotype thus includes a heterogeneous group of smokers, ranging from people who may have tried smoking cigarettes only once, to heavy, dependent cigarette smokers. Not surprisingly, estimates of genetic and shared environmental effects on smoking initiation vary greatly across studies. Some studies have shown greater importance of shared environmental effects (44% to 54%) compared with genetic effects (11% to 39%) on smoking initiation;[33,113,123–125] other studies have shown substantial heritability (43% to 85%) and a relatively smaller role of shared environment (0% to 68%) for smoking initiation.[33,98,117,120,121,126–128] Conceivably, studies that report greater genetic effects for smoking initiation may contain a greater proportion of regular and heavier smokers—two smoking dimensions with a strong genetic signal, relative to occasional or lighter smokers—for whom genetic differences from nonsmokers may be less important than environmental. Also, evidence shows differences in the relative contribution of genetic and shared environmental effects for smoking initiation across gender, age and age cohort, and culture.[33,120,121,123,126]

At the next level of measurement, most measures of nicotine dependence, such as the FTND and the *DSM*-based classification, have shown acceptable levels of test-retest reliability, and some have been reported to have high to moderate heritability. The validity of many of the measures, however, is uncertain because the two primary measures of nicotine dependence are not correlated with each other, and the extent to which nicotine dependence is associated with motivations, withdrawal, and/or smoking topography is generally unknown as well.

For class II nicotine-dependence phenotypes, defined by the *DSM* Third Edition Revised,[129] *DSM-IV,*[63] or Fagerström criteria, heritability estimates ranged from 31% to 60%,[65,88,89,128] and for dependence as defined by the Heaviness of Smoking Index (HSI),[130] which comprises two of the seven FTND items, heritability was also high (59% to 71%).[65,89] In an analysis examining the genetic relationship between lifetime regular smoking and nicotine-dependence operationalization by using items from the FTQ[53] and the *DSM-IV,*[63] Kendler and colleagues[98] found substantial genetic effects for regular smoking (85%), substantial overlap in liability for regular

smoking and nicotine dependence (60%), and moderate residual genetic effects for nicotine dependence (22%), suggesting overall considerable heritable influences on both regular smoking and nicotine dependence. Individual diagnostic criteria also have been shown to be significantly heritable (26% to 73%), with little to no evidence for a significant contribution from shared environmental effects.[65,89,131] Individual nicotine withdrawal symptoms have been shown to be moderately heritable, ranging from 9% to 53%,[89] with no evidence for a significant contribution from shared environmental effects.

Class III phenotypes—or smoking trajectories—appear to be reliable across a number of studies, although the extent to which they can be assessed reliably with a retrospective methodology is not clear. Their validity as constituents (precursors) of adult nicotine dependence is unknown as is the extent to which membership in a trajectory subgroup is influenced by genetic factors. Before the appearance of the present volume, no studies had been published on the contribution of genetics to variation in longitudinal tobacco use phenotypes (class III), such as developmental smoking trajectories. One study of adolescent twins that involved three data collection periods across seven years generated cross-sectional smoking groups (never smokers, triers, experimenters, current smokers). The study examined the cross-sectional heritability of a smoking index variable that combined frequency and recency of cigarette smoking across groups and thus captured the smoking experience across groups at each time of assessment, but not the smoking experience of each group across assessments.[19] The study found that, at each wave of data collection, the smoking index variable was significantly influenced by genetic factors (21.8% at wave 1, 22.8% at wave 2, and 35.5% at wave 3) and by shared environmental factors (52% at wave 1, 51.7% at wave 2, and 36.7% at wave 3).[19] Of note is the importance of shared environmental factors which, while not measured in this study, include influences such as protobacco advertising and product availability. The first investigation of twin concordance for tobacco- and alcohol-use trajectories is presented in chapter 7.

Several studies have examined the relative contribution of genetic and environmental influences on the transitions from smoking experimentation to higher levels of smoking and nicotine dependence. Using correlated liabilities models, these studies have shown an overlap in the genetic and environmental influences on liability to smoking initiation with liability to smoking persistence,[33,117,120] smoking quantity,[113] regular use,[127] and nicotine dependence.[98,127] At least two studies demonstrated that, in older age groups (aged 30 years and older), genetic and environmental factors that determine liability to smoking initiation are independent from those that determine liability to smoking persistence.[33,120] Except for one study,[127] these studies showed a relatively larger influence of shared environmental factors on smoking initiation and a smaller to no significant influence of shared environment on smoking persistence, quantity smoked, or nicotine dependence, consistent with much other work, as discussed earlier. While not measured in the present study, shared environmental influences could include the well-documented effects of smoke-free homes.

Finally, while the measurement properties of some of the class IV phenotypes such as nicotine pharmacokinetics and dynamics are well described, and significant heritability has been demonstrated in both biometric and measured genetic contexts (see the section below, "Heritability of Nicotine Metabolism," for an example), these characteristics for many of the other potential candidate endophenotypes

are unknown. Moreover, the validity of these measures as constituents of nicotine dependence appears thus far to be problematic or without documentation.

The majority of adolescent and adult twin studies agree that the relative contribution of genetic influences on smoking initiation is smaller than that on downstream smoking phenotypes such as progression to regular smoking, smoking persistence, nicotine dependence, and smoking cessation. Conversely, the relative contribution of environmental factors is larger in smoking initiation than in downstream smoking phenotypes. These results suggest that environmental factors play an important role in experimentation with cigarettes, which largely occurs in adolescence, and that, beyond a certain level of experimentation, genetic liability becomes a stronger determinant of cigarette smoking. From the perspective of intervention and genetic studies of smoking, it appears that a genetically informative endophenotype for nicotine dependence may vary across age, as well as across levels of use.

With regard to existing molecular genetic literature involving smoking-related phenotypes, most reported studies examined class I phenotypes measured retrospectively.[48,132–152] Since 2003, the number of papers that use retrospective measures of nicotine dependence—that is, class II indices—has been increasing.[142,153–167] Retrospective case-control designs are subject to limitations of recall bias. Many of these studies have not received independent confirmation as of the writing of this chapter. A notable exception to the use of retrospective self-report measures of nicotine dependence is the paper by Ray and colleagues,[168] in which an experimental measure of the relative reinforcing value of nicotine (a class IV phenotype) was found to be associated with variation in the gene *OPRM1*.

Genetic Epidemiological Concepts and Their Implications for Studying Nicotine Dependence

Characteristics of Complex Genetic Traits

Nicotine dependence, a multidimensional construct, is a complex genetic trait. In this context, the term *complexity* is used as defined by the field of genetic epidemiology.[169–172] A complex trait has several defining features: (1) it has reduced penetrance (i.e., not everyone with a susceptibility gene[s] will develop nicotine dependence); (2) genetic heterogeneity is involved (i.e., a different set of susceptibility genes may contribute to the likelihood of becoming a smoker in different people); (3) pleiotropy is involved (i.e., the same genetic risk factors may lead to different addictions, such as alcoholism, in addition to nicotine dependence in different people); (4) epistasis is involved, which refers to the situation in which a genetic risk factor modifies the expression of another genetic risk factor to produce nicotine dependence; and (5) environmental factors can interact with genetic risk to alter the likelihood of becoming dependent on nicotine.

The available literature involving measured genetics and nicotine dependence provides ample evidence that it is, indeed, a complex genetic trait. Incomplete penetrance is demonstrated by the fact that heritability of nicotine dependence is roughly 50% and that the odds of being a smoker even in the presence of a genetic risk factor is, on average, higher than in the absence of the risk allele.

Genetic heterogeneity is clearly evident when considering the number of genes that have been reported to be in association with nicotine dependence. These include *CHRNA4,*[155,156] *CHRNA7,*[142] *CHRNA9,*[142] *CHRNB1,*[164] *CHRNB2,*[142] *CHRNB3,*[142] *OPRM1,*[168,173] *DRD2* (evidence, however, suggests that the association with *DRD2* may be confounded by close proximity to *ANKKI,* a kinase gene),[146,174] *DRD4,*[134] *COMT,*[159] *SLC6A3,*[135] *5-HTTLPR,*[133] *5HT2A,*[147] *CYP2A6,*[29,150,163,175] *CYP2E1,*[161] $GABA_{B2}$,[158] *MAOA,*[148,152,162] *TH01,*[149,153,154] *TPH,*[166] *SLC18A2,*[151] *PTEN,*[157] *NTRK2,*[159] *EPAC,*[160] *DDC,*[165,167] *CHRM1,*[164] *CCK,*[176] and *BDNF.*[177] A close review of these papers indicates substantial variation in the nature of the phenotype measured, ranging from CPD, maximum CPD, nicotine dependence, heavy smoking, smoking status, smoking initiation, withdrawal, regular smoking, and the relative reinforcing value of nicotine. Going forward, what is the best way to incorporate genetic heterogeneity into studies of nicotine dependence? What, if anything, should be concluded about the fact that the published linkage studies have identified regions for the most part that do not contain the candidate genes of interest? One answer to the problem of genetic heterogeneity is the use of an appropriate statistical framework capable of incorporating information about numerous genetic variants while simultaneously taking into account previous findings and expert knowledge to make sense of the plethora of associations reported. The use of Bayesian hierarchical modeling as informed by an ontological framework is described in chapter 12.

Pleiotropy is apparent because genes such as *DRD2* have been reported as associated with other addictive behaviors and/or affective disorders. That any one single gene may, in fact, be associated with a number of phenotypes is an issue that requires much more attention in the literature. In addition to being associated with smoking-related phenotypes such as ever smoking,[178] smoking cessation in response to acupuncture,[179] smoking cessation in response to bupropion,[180] and smoking cue-induced cigarette craving,[181] variation in *DRD2* has been reported as being associated with schizophrenia,[182–184] alcoholism,[185,186] quantity of alcohol consumed by adolescents and young adults,[187] obsessive compulsive disorder,[188] ADHD,[189,190] cue-elicited craving for heroin,[191] comorbid depression, anxiety, and social dysfunction associated with posttraumatic stress disorder,[192] working memory in schizophrenics,[193] Tourette's syndrome,[194] anorexia nervosa,[195] neuroticism/anxiety in men,[196] and opium addiction.[197]

The range of phenotypic correlates suggests, at the least, that variation in *DRD2* is not specific to nicotine dependence. The extent to which these indices of psychopathology are viewed as covariates or confounders of the association between nicotine dependence and variation in *DRD2* is highly variable across the published papers on this relationship. Another issue lacking clarity in the literature is the extent to which this plethora of psychiatric phenotypes is associated with and/or has any phenotypic subcomponent in common with nicotine dependence. Similar questions of phenotypic covariation can be raised about the literature involving variation in *OPRM1, 5HTT, MAOA,* and *CHRNA4.* Evidence is addressed in chapter 8 that some of these phenotypes may serve as early indicators of risk for nicotine dependence before chronic exposure to nicotine.

Epistasis, the interaction between genes, was first reported by Lerman and colleagues[139] for *DRD2* and *SLC6A3* and then again by Lerman and colleagues[198] and by Swan and others.[199] These studies underscore the importance of considering the simultaneous effect of several genes on behavior in that the observed effect of any one gene may strictly depend on variation in another gene. For example, investigation of the effect of

genes that are part of a common neural pathway that underlies behavior may be an effective approach (chapter 12 discusses the need for pathway analyses in greater depth).

No published examples of gene-environment interactions in nicotine dependence were found in the literature. Emerging work in the psychiatric genetic literature provides an example that such interactions may exist. The work of Caspi and colleagues[200] reveals that variation in the *5HTT* gene may moderate the impact of life stress on depression. The subsequent work of Kendler and others[201] supports Caspi's original findings and extends them by demonstrating that the threat level of the life stress may be the most critical aspect in interaction with *5HTT* to ultimately produce depression.

The conventional twin model has been extended to account more fully for the effects of gene-environment interactions and/or correlations. Purcell[202] provides the tools to extend the traditional twin model to include a component for the effects of a moderator variable which, in the present case, could be a measure of the environment. Purcell indicates that, while having both genes and environment as measured variables would provide the most power to detect a gene-by-environment (G×E) interaction, as in Caspi and colleagues,[200] most modern twin studies should be able to rely on a latent, unmeasured G and a measured E. The most powerful approach to the measurement of E will be a continuous measure. The application of these models[202–204] has been demonstrated by Button and colleagues,[205] who showed that the heritability of antisocial scores in young twins declines as family dysfunction scores increase, and by McCaffrey and others,[206] who examined the relationship between education level and nicotine dependence in twins. The evidence that macrocontextual (e.g., cultural and socioregional) factors can modify genetic effects is reviewed in chapter 11 of this volume. New approaches to the assessment of microcontextual (e.g., parental and peer smoking) factors are also described.

Implications for Selection of Nicotine-Dependence Phenotypes and Endophenotypes

Are Multiple Nicotine-Dependence Phenotypes Associated with Each Other?

As discussed in chapters 3 and 4, the issue of construct validity is of major importance to the pursuit of knowledge in this area. The extent to which various nicotine-dependence phenotypes are or are not associated with each other or with a universally accepted gold standard of nicotine dependence has not been well studied in the literature. For example, while a measure of consumption such as CPD may be highly correlated with the total FTND score ($r > 0.60$), a measure of nicotine metabolism, considered to be a basis for dependence, is correlated only modestly with CPD ($r = 0.12$, $p < 0.05$;[207] $r = –0.15$, p is not statistically significant;[208] $r = 0.33$, $p < 0.01$;[209] and not at all with the FTND).[207,209–211] Similarly, while adolescent trajectories of tobacco use can be clearly demarcated on the basis of number of cigarettes smoked, the extent to which adolescent nicotine metabolism is associated with trajectory group membership is unknown. The first evidence that trajectory group membership in adolescence may be associated with adult nicotine dependence is presented in chapter 5.

Are Multiple Nicotine-Dependence Phenotypes Associated with a Single Endophenotype?

The relationships that exist between each "marker" of dependence within each phenotypic domain need to be determined, along with the relationships that exist

across phenotypic domains, to reach a comprehensive understanding of the nature of nicotine dependence. For example, a long-standing hypothesis states that the rate of nicotine metabolism should be related to smoking behaviors, with faster elimination of nicotine being associated with increased rates of smoking and nicotine dependence.[25] While there are few published tests of this hypothesis, the papers that have been published lend only limited supporting evidence, with the rate of nicotine metabolism accounting for less than 16% of the variation in CPD[209,210] and no significant amount of variance in the FTND[209–211] or in the Horn-Russell Scale.[210] Kandel and colleagues[211] found no significant association between the rate of metabolism and CPD in a sample of younger, lighter smoking, and less dependent smokers. A review of the discussion of results from these papers offers the following possible reasons for the apparent disconnect between rate of metabolism and nicotine dependence: (1) the questionnaire measures of adult nicotine dependence used may not be the most sensitive measures of rate of metabolism,[209,210] (2) the rate of metabolism may only be related to nicotine dependence during the transition from experimentation to "addicted" smoking,[209] or (3) the rate of metabolism is not an important determinant of smoking behavior in younger smokers because of a low level of smoking.[211]

From the standpoint of the present volume, the lack of evidence that the rate of nicotine metabolism is an important driver of nicotine dependence should create some urgency as to its construct validity. On one hand, the rate of metabolism is associated (although weakly) with CPD. CPD, at the same time, is substantially correlated with most or all existing measures of nicotine dependence. While the resolution of the apparent logical inconsistencies in the literature is beyond the scope of this chapter, some suggestions are offered for future research. For example, is "time to first cigarette after waking up"—one of the key components of the FTND—associated with nicotine metabolism? One would hypothesize that individuals with faster clearance of nicotine or more extensive conversion of nicotine to cotinine would be associated with a shorter time to the first cigarette. Again, using nicotine metabolism as an endophenotype, is variation in nicotine clearance associated with subjective reactions to the first cigarette of the day? Chapter 3 makes a strong case for developing a comprehensive theory of nicotine dependence as a way to understand apparent logical inconsistencies in research findings.

Are Multiple Nicotine-Dependence Endophenotypes Associated with a Single Phenotype?

Another set of addressable questions emerge when the relationship among endophenotypes is considered. For example, is variation in nicotine metabolism related to performance increases on the measure of executive function or to related nicotine reward? If metabolism and executive function are related, are they associated to the same degree with specific and global measures of nicotine dependence? Chapters 8 and 9 suggest that relatively little is known about the relationship between candidate endophenotypes and measures of nicotine dependence.

Why Are Environmental Phenotypes Important?

Given the success of policy interventions in reducing smoking rates, some have argued that resources invested in genetics research on smoking would be better spent on those intervention strategies. The reasoning is that nicotine dependence "appear[s] to be highly amenable to environmental modification," and "[r]esources would be far better placed in designing effective interventions and studying the causes of the gap between

knowledge and modification of health-related behaviors."[10(p601)] This argument, however, rests on a false dichotomy between the roles played by genes and environment in the etiology of nicotine dependence.

As argued in rebuttal to the above viewpoint,[12,212,213] contemporary genetic research of complex diseases takes into account both genes and environment and seeks practical results within the full scope of etiologic mechanisms. The environment (e.g., aggressive tobacco promotions, cigarettes designed to maximize their addictive potential), rather than genes, is the most likely target of intervention. Moreover, the results of some molecular genetic studies of behavioral disorders[214,215] have shown that genetic information may be critical to the discovery of environmental effects and vice versa.

Despite the fact that the genetic mechanisms of many genetic disorders are already known, this has not necessarily translated into efficient interventions exactly for the reason that these mechanisms are difficult to change. Genetic studies of complex disorders, in contrast, have barely departed from their nascent stage, but the significant contribution of environmental factors, even in the natural variation in the risk, promises a greater payback. It has long been understood that, regardless of heritability, the individual genotype determines the range of possible phenotypes under possible environments, the norm of reaction.[216]

Numerous environmental risk factors for acquiring nicotine dependence have been identified in the literature.[217] However, it is not yet clear whether any of these have the possibility of interacting with genetic risk factors to heighten the likelihood that an individual will become dependent. A number of these have the potential to be an "environmental pathogen"—that is, a characteristic of the environment in the presence of which a genetic risk factor can exert its effect on nicotine dependence.[218] One of the challenges in this area is the need for optimal measurements of the environment so that proximal and distal risks can be enumerated, along with the documentation of age-specific and cumulative risk. Moffitt and colleagues[218] identify a strong need for improved retrospective measurement of environmental pathogens (see chapters 3 and 11 for further discussion of environmental pathogens and their measurement).

Evidence suggests that a portion of the smoking population smokes every day, has not previously attempted to quit, and has no desire or intention to quit. Prevalence estimates for this "hard-core" smoking range from 5% to 16% of the smoking population.[219–221] Further characterization of hard-core smokers indicates several characteristics shown to have a genetic component (e.g., shorter time to first cigarette of the day, heavier smoking, concurrent use of other tobacco products, use of other abused substances, and comorbid depression). Given that hard-core smokers tend to be of lower socioeconomic status and are more likely to be unemployed and living alone, Warner and Burns[222] have speculated that these types of smokers may be living in a more stressful environment. Interestingly, stress reactivity has been shown to have both genetic[223,224] and environmental[225] components in its variation. This raises some interesting questions about the hard-core smoker that should be addressed in future research: (1) Is the prevalence of certain candidate gene variants higher or lower in hard-core smokers? (2) Does the relationship between specific candidate gene variants and hard-core smoking vary as a function of exposure to certain environmental risk factors? (3) Is the constellation of genetic and environmental risk factors different in hard-core smokers? (4) Are there subgroups within the hard-core smoker population that vary in genetic and environmental risk

factors? Genetic epidemiology investigations of this group of smokers may provide a wealth of information to inform future tobacco control efforts in hard-to-reach segments of the smoking population.

Could Environmental Variation Cause Variation in Expression of Genes of Relevance to Nicotine Dependence?

The evidence is compelling that environmental factors can result in the expression of genes in pathways of relevance to addiction in general. For example, exposure to stress modulates the expression of cocaine- and amphetamine-regulated transcript *(CART)* in the hypothalamus and amygdala in the rat brain in a region- and sex-specific manner. *CART* may, therefore, be a mediator peptide in the interaction between stress and drug abuse.[226] In a series of studies, early maternal care was shown to have a profound impact on gene expression with long-lasting effects on the stress response.[227,228] Chronic stress influences gene transcription in the hippocampus.[229] Differential exposure to enriched or impoverished environments alters *N*-methyl-D-aspartate receptor subunit expression in the nucleus accumbens core and shell.[230] Also, exposure to drugs of abuse (e.g., opiates) results in a discernible pattern of gene expression in the opioidergic and other pathways.[231–233]

Epigenetics refers to heritable variation in biochemical modifications of both the nucleic acid and the protein components of chromatin—that is, the methylation of cytosine found in cytosine-guanine dinucleotides and posttranslational modification (methylation, acetylation and phosphorylation) of histone proteins, generally associated with decreased or increased levels of gene expression at the corresponding genetic locus.[234] Multiple approaches to the analysis of epigenetic variation and its associations with genetic and environmental variation and their association with disease are possible. One elegant design, however, uses monozygotic twins. These twins share 100% of their genome at the moment of twinning and accumulate differences thereafter with respect to DNA methylation, histone modification, and copy-number variation.[235,236] Age, diet, gender, and environment have been associated with global epigenetic modification of the genome and both global and locus-specific DNA methylation appear to be heritable.[236–239] While both genetic[240] and epigenetic[234] variation are associated with individual differences in gene expression, the discordant monozygotic twin design may be the most promising design to investigate epigenetic regulation of gene expression that might underlie differences in a phenotype of interest.[241]

The study of discordant monozygotic twin gene expression or epigenetic differences is still in the early stages with respect to the numbers of studies, numbers of individuals, and design characteristics. A review of the literature reveals nine investigations of gene expression differences between discordant monozygotic twins that evaluate a panel of genes for gene expression differences to identify candidate genes potentially associated with the discordant phenotype,[242–249] with the number of twin pairs evaluated in these studies ranging from 1 to 11. Five of these studies have used lymphoblastoid cell lines as the tissue source,[242,243,245,249,250] two studies include analysis of dizygotic twin pairs or siblings in their gene expression analyses,[243,248] and five studies used the Affymetrix gene expression array platform.[244–246,249,250] Four studies confirmed specific results using individual candidate gene expression analysis in the discovery sample of RNA from the discordant monozygotic twins,[245,248–250] and one study validated specific gene expression results in a second RNA sample derived from sporadic cases and controls.[249]

The use of monozygotic twins discordant for smoking history or nicotine pharmacokinetics for epigenetic studies to identify candidate genes influencing these traits represents a complementary approach to candidate gene and genome-wide association studies (GWAS). Candidate genes identified using these approaches can then be evaluated directly for specific epigenetic differences in genomic DNA from the discovery sample of discordant monozygotic twins and in genomic DNA from other individuals for association to the traits of interest. The availability of public epigenetic data and additional research into the prevalence and correlates of epigenetic modifications[251] will enable the data from such twin design epigenetic analyses to be placed within the population of genetic, environmental, and genomic and epigenomic contexts.

Summary of Selected Biometric and Measured Genetic Studies of Nicotine Dependence

The following studies are summarized below as examples of biometric and measured genetic studies of nicotine dependence. The reader will see a progression from completely biometric analyses of components of nicotine dependence (class II phenotypes),[65] biometric and measured genetic analyses combined (class IV phenotypes),[252] to measured genetics with a range of class I and II phenotypes,[66] and, finally, many measured genetic variants in relation to one class II phenotype.[13,253] The studies are included as representative of the state of the science involving genetics and nicotine dependence. The reader will see, however, that none of the examples address issues raised in the present volume concerning theoretical and measurement issues of nicotine dependence and, therefore, set a baseline from which future studies should progress.

Heritability of Components of Nicotine Dependence in Adults

A study in 2004 identified genetically informative nicotine-dependence criteria in a large community sample of adult (aged 24–36 years) Australian male and female twins.[65] The phenotypes under investigation were the seven *DSM-IV* nicotine-dependence criteria[63] and two FTND items (CPD and time to first cigarette in the morning) that together make up the HSI.[130] In the first step of the analysis, the phenotypic factor structure of the nine nicotine-dependence criteria in ever smokers resulted in two highly correlated factors for both women and men, with items related to smoking quantity loading on the first factor (*DSM-IV* nicotine tolerance and both HSI items), and *DSM-IV* items related to withdrawal and difficulty in quitting smoking loading on the second factor (withdrawal, smoking more than intended, difficulty in quitting smoking cigarettes, giving up important activities to smoke, and smoking despite physical or psychological problems caused by or exacerbated by smoking). Chain smoking, corresponding to the *DSM-IV* criterion of a great deal of time spent using the substance loads equally strongly on both factors and, in exploratory analysis, loaded highly on a third factor for both women and men, suggesting that this item does not correlate with endorsement of the remaining items. Internal consistency was high for both factors in women and men (Cronbach's alpha ranged from 0.78 to 0.79).

Factor analysis suggested similarity in the pattern of endorsement of nicotine-dependence criteria in women and men. Further, the weak factor loading of time to first cigarette in the morning on the factor for which withdrawal had a strong loading suggested that latency to first morning cigarette does not index nicotine withdrawal.

Finally, the *DSM-IV* criterion of giving up important social and occupational activities to smoke had the weakest correlation with the total factor score. Factor internal consistency improved without this item in both women and men. This result, together with this item being the least commonly endorsed, suggests that giving up activities to smoke may not be an important indicator of nicotine dependence in adults.

Genetic factor analysis of the same nine nicotine-dependence criteria resulted in two genetic factors and one shared environmental factor for both women and men. High item loadings were observed for all items on the first genetic factor with weaker loadings on the second genetic factor, suggesting that similar genetic factors contribute to interitem correlation. Factor loadings on the second genetic factor were opposite in sign in the women compared to the men, implying the influence of different genetic factors. Factor loadings on the shared environmental factor were low in women and moderate in men, and were opposite in sign between women and men, suggesting gender differences in the shared environmental factors that contribute to interitem correlation.

A study by Lessov and colleagues[65] also examined the relative contribution of genetic and environmental influences on variance to individual nicotine-dependence criteria and a nicotine-dependence diagnosis as defined by the *DSM-IV* and the HSI. The results showed substantial heritability for *DSM-IV* nicotine tolerance (73%), withdrawal (53%), smoking more than intended (62%), and both HSI items—time to first cigarette in the morning (68%) and number of CPD (70%). Relatively moderate heritability was observed for *DSM-IV* items: ever chain smoked (45%), smoking despite physical or psychological problems (39%), and giving up important activities to smoke (26%). There was no evidence for a significant contribution by shared environmental effects for any of these items and no gender differences.

One exception was the *DSM-IV* criterion of difficulty in quitting smoking, which was strongly heritable in women (68%), with no significant contribution for shared environment, and relatively more weakly heritable in men (54%), with significant contribution of shared environmental effects (26%). Nicotine dependence defined by *DSM-IV* criteria (i.e., endorsing three or more of seven items in the same 12-month period lifetime) was moderately heritable (56%); higher heritability was observed for HSI-defined dependence (71%). For both dependence definitions, there was no evidence for significant shared environmental effects or gender differences.

Taken together, the results from the Lessov and colleagues study[65] suggest that the *DSM-IV* criteria of giving up important activities to smoke and chain smoking (i.e., spending a lot of time using nicotine) may not be useful indicators of nicotine dependence for the purpose of genetic research. However, the *DSM-IV* criteria of tolerance, withdrawal, and difficulty in quitting smoking, and the two HSI items—time to first cigarette in the morning and CPD—may be the most salient genetic indicators of nicotine dependence in adults. The results also suggest that factor analytic approaches may identify highly genetically informative dependence phenotypes. Future work will examine the phenotypic and genetic factor structure of nicotine dependence in adolescents, which could be expected to be different from that of adults, considering differences in the importance of social and cultural pressures in relation to cigarette smoking.

Heritability of Nicotine Metabolism

The twin design has been used previously to investigate the genetic and environmental variance of the metabolism of a variety of substances including ethanol, lithium, and halothane (an anesthetic), but its

application to the study of nicotine metabolism did not begin until 2004. Although the body of evidence from the early twin studies shows substantial genetic involvement in drug metabolism, the extant literature has (1) examined relatively few pharmacokinetic indices of drug metabolism, (2) relied on very small samples of twins, (3) not used state-of-the-art techniques for quantification of the relative contribution of genetic and environmental influences, and (4) been unable to examine the impact of measured P450 genotype on estimates of broad heritability.

In a series of papers, the adaptability of the twin design to a variety of purposes was demonstrated.[252,254–259] When combined with methodologies from molecular genetics, the design becomes highly informative with regard to the impact of measured genotype on estimates of heritability when the family nature of the data is used, as well as the impact of genotype on the metabolic phenotypes when the data are treated as coming from unrelated individuals.

Although certain genes for enzymes, such as *CYP2A6,* are clearly implicated in relevant pathways for nicotine metabolism,[26,27,260] development of a complete understanding of all relevant candidate genes (e.g., *CYP2B6*[261] and *CYP2D6*[262,263]) and their interactions in the pathways is still under way. For the purposes of the present example, the focus is only on that portion of the metabolic pathway that involves principally the action of *CYP2A6* in the conversion of nicotine to cotinine.[252] One hundred and thirty-nine twin pairs—110 monozygotic and 29 dizygotic—underwent a 30-minute infusion of stable-isotope-labeled nicotine and its major metabolite, cotinine, followed by an 8-hour in-hospital stay. Blood and urine samples were taken at regular intervals for analysis of nicotine, cotinine, and metabolites by gas chromatography–mass spectrometry or liquid chromatography–mass spectrometry and subsequent characterization of pharmacokinetic and metabolism phenotypes. DNA was genotyped for zygosity and for variation in the gene for the primary enzyme involved in nicotine metabolism, *CYP2A6* (alleles tested: **1, *1/2, *2, *4, *7, *9,* and **12*).

Standard pharmacokinetic parameters were estimated from blood concentration data by using model-independent methods as described elsewhere.[264,265] Univariate genetic analyses were used to quantify the relative contribution of genetic and environmental influences. All analyses were adjusted for age, current smoking, and oral contraceptive use in women.

Approximately 60% of the variability in clearance of nicotine, and clearance of nicotine via the cotinine pathway, was attributable to additive genetic effects. The estimate of additive genetic variation in the clearance of cotinine was smaller (33.3%). All three clearance parameters were significantly faster in the *CYP2A6* wild-type homozygous participants compared with those with at least one reduced metabolizing gene variant.

It was hypothesized that the estimate of additive genetic influence on measures of clearance would be reduced after adjusting for the effects of the *CYP2A6* genotype. The effect of measured *CYP2A6* genotype was tested by (1) including genotypic status (wild-type homozygotes or the presence of at least one reduced metabolizing variant) as a covariate in the genetic models and estimating the relative contribution of genetic and environmental effects to the residual phenotypic variance and (2) fitting models to metabolism data after excluding all individuals with at least one *CYP2A6* variant.

The inclusion of the *CYP2A6* genotype as a covariate in the biometric models did not significantly alter the estimate for additive genetic effects on the three measures of clearance. As hypothesized, point estimates

for the additive genetic effects decreased, but only to a small degree (e.g., decreases of 7.7%, 9.0%, and 7.4% for clearance of nicotine, clearance of cotinine, and clearance of nicotine, via the cotinine pathway, respectively). Similarly, exclusion of individuals with reduced metabolizing allele variants of *CYP2A6* resulted in best-fitting models with decreased but still significant heritability for the residual phenotypic variation in the clearance measures (e.g., decreases from point estimates for the sample as a whole of 8.8%, 26.4%, and 14.8% for clearance of nicotine, clearance of cotinine, and clearance of nicotine, via the cotinine pathway, respectively). These results suggest that, to the degree that twin variation in these nicotine clearance parameters is attributable to variation in *CYP2A6* allele status, the effect is small and that genetic influences in addition to variation in *CYP2A6* contribute to the heritability of nicotine and cotinine clearance (see chapter 12 for an analysis of nicotine metabolism involving multiple genes). The small association between the *CYP2A6* genotype and clearance measures, both at the phenotypic and genotypic levels, may partly explain why the relationship between the *CYP2A6* genotype and smoking behavior is inconsistent.[51,52]

In addition, this study by Swan and colleagues[252] tested relatively few *CYP2A6* variants (i.e., those known or very likely to have an impact on the structure of the gene and resulting protein). New variants are appearing at a very rapid rate, and many are not yet characterized or numbered.[266] The majority of these variants are in the 5′ and 3′ noncoding regions, which some studies have found to alter levels of transcription.

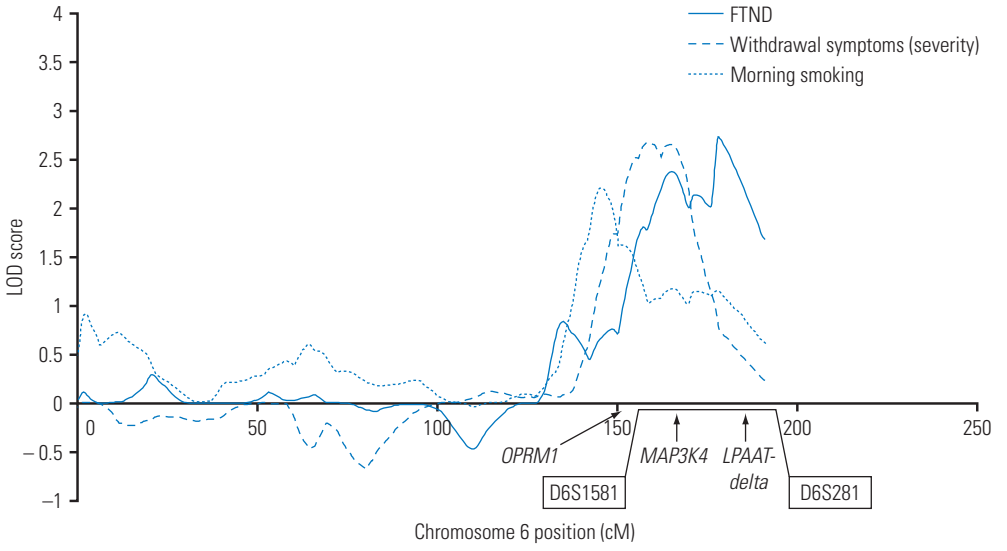
Linkage Analysis of FTND and Other Indices of Nicotine Dependence

The family study by Swan and others[66] sought to identify loci that segregate with nicotine dependence as determined by the FTND. Additional measures were included to capture the complexity of the nicotine-dependence phenotype.[66] These included: (1) elements from the *DSM-IV* dependence criteria,[63] (2) smoking frequency and quantity, and (3) quitting history. Individuals who never tried even a puff of a cigarette were excluded from all definitions. Individuals who tried smoking, and those who smoked 100 or more cigarettes in their lifetime but were never daily smokers, are included in the zero category of *DSM-IV* measures. All other measures included lifetime daily smokers only.

For the FTND summary score, a maximum logarithm of odds (LOD) score of 2.7 was seen at 178 cM on chromosome 6 (figure 2.3). The marker closest to the peak was D6S446. The support interval (defined as the region in which LOD scores are within the value of one less than the maximum LOD score) included 156–191 cM (D6S1581-D6S281). In subsequent analyses, additional tobacco use phenotypes were examined for evidence of linkage. To minimize the reporting of results due to chance, individual LOD scores of 2.7 or greater only were reported. The support interval for withdrawal severity overlapped the FTND support interval on chromosome 6 (figure 2.3) with a peak LOD score of 2.7. Also shown in figure 2.3 is a quitting-history phenotype, short-term quit, that had a peak LOD score of 1.9 in the same region. The largest LOD score for any nicotine-dependence phenotype (LOD score = 3.0) was observed for *DSM-IV*-like nicotine-dependence severity near D7S636 (164 cM; support interval 159–167 cM; figure 2.4). For the dichotomous *DSM-IV*-like nicotine-dependence measure, a maximum LOD score of 2.7 was observed on chromosome 8 at 31 cM and 35 cM (near marker D8S258; figure 2.5).

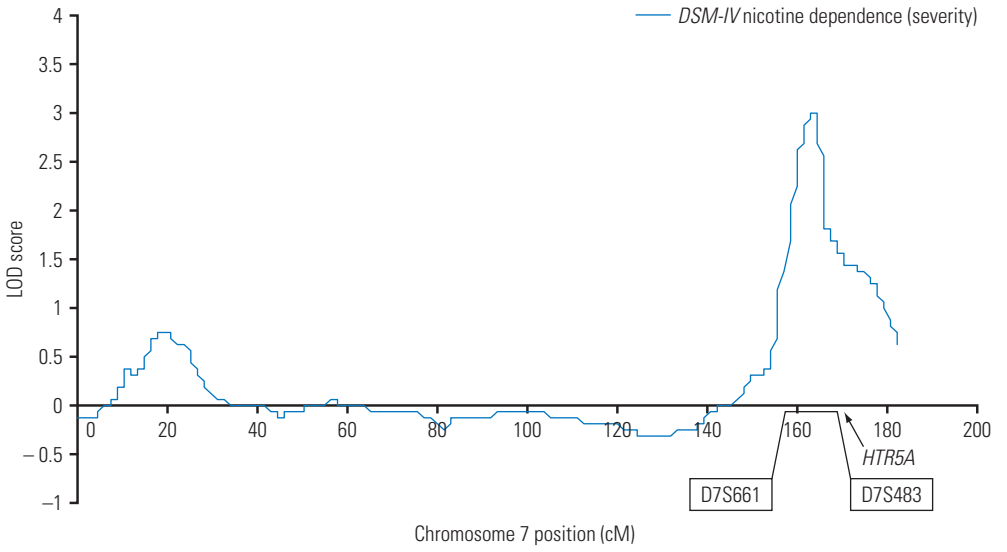
Previous work has identified linkage peaks at or near the support interval reported

Figure 2.3 Multipoint Linkage Plot—Chromosome 6



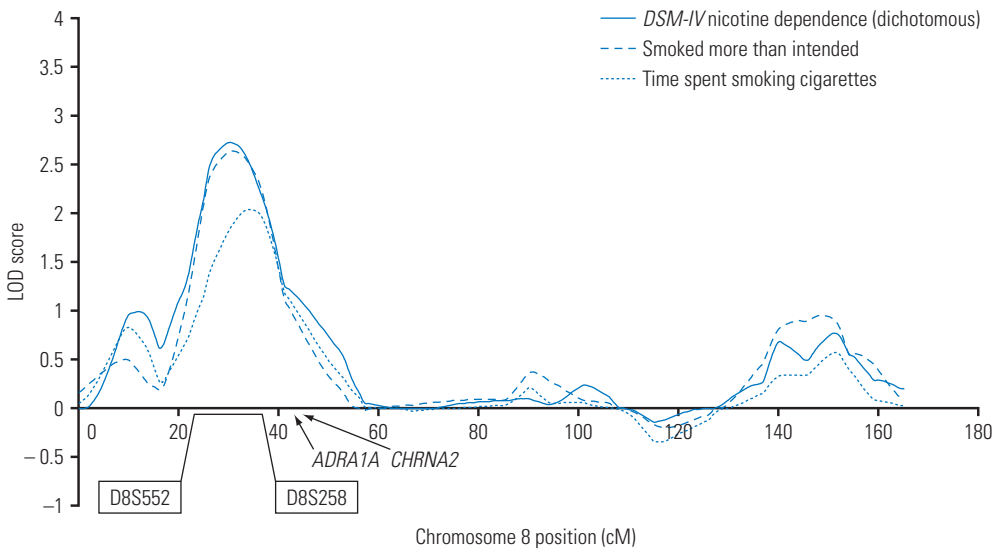
Note. FTND = Fagerström Test for Nicotine Dependence; LOD = Logarithm of odds; cM = centimorgans; Bergen and colleagues[267] (1999; ever smoke) and Sullivan and colleagues[55] (2004; Fagerström Tolerance Questionnaire) have peaks at beginning of support interval. From Swan, G. E., H. Hops, K. C. Wilhelmsen, C. N. Lessov-Schlaggar, L. S. Cheng, K. S. Hudmon, C. I. Amos, et al. 2006. A genome-wide screen for nicotine dependence susceptibility loci. *American Journal of Medical Genetics Part B, Neuropsychiatric Genetics* 141 (4): 354–60.

Figure 2.4 Multipoint Linkage Plot—Chromosome 7



Note. DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; LOD = logarithm of odds; cM = centimorgans. Sullivan and colleagues[55] (2004; Fagerström Tolerance Questionnaire) have a peak near the start of the support interval. From Swan, G. E., H. Hops, K. C. Wilhelmsen, C. N. Lessov-Schlaggar, L. S. Cheng, K. S. Hudmon, C. I. Amos, et al. 2006. A genome-wide screen for nicotine dependence susceptibility loci. *American Journal of Medical Genetics Part B, Neuropsychiatric Genetics* 141 (4): 354–60.

Figure 2.5 Multipoint Linkage Plot—Chromosome 8



Note. DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; LOD = logarithm of odds; cM = centimorgans. Bergen et al.[267] (1999; ever smoke) reported three significant peaks within support interval. From Swan, G. E., H. Hops, K. C. Wilhelmsen, C. N. Lessov-Schlaggar, L. S. Cheng, K. S. Hudmon, C. I. Amos, et al. 2006. A genome-wide screen for nicotine dependence susceptibility loci. *American Journal of Medical Genetics Part B, Neuropsychiatric Genetics* 141 (4): 354–60.

here for FTND.[55,267] Moreover, the support interval is very close to the *OPRM1* gene and contains *MAP3K4* and *LPAAT-delta,* both candidate genes for nicotine dependence.[55] It is encouraging that several loci reported here have been detected in other linkage studies as well. The support interval on chromosome 7 observed here for *DSM-IV*-like nicotine-dependence severity is near the linkage peak, D7S1804, reported previously for the FTQ,[55] and is near the candidate gene *HTR5A.* The support interval seen in the present study on chromosome 8 for *DSM-IV*-like nicotine dependence is near previously reported linkage peaks for the ever-smoking phenotype[267] and is close to the candidate genes *CHRNA2* and *ADRA1A.* Whether the heterogeneity across chromosomes for indices of nicotine dependence derives from genetic or measurement sources cannot be determined from the present study and needs to be addressed in future research.

Genome-wide Association and Candidate Gene Studies of FTND

Smoking initiation occurs with the experimentation and use of cigarettes, often in adolescence. After smoking 100 or more cigarettes, a person passes the threshold to become a "smoker," the term used in most health and population-based surveys. Various behaviors are seen among smokers, ranging from low-level cigarette use by "chippers" to heavy smoking by nicotine-dependent individuals who have difficulty quitting. Different factors contribute to the transition from one smoking level to the next, including genetic and environmental factors. Some of the risk and protective factors that play a role in smoking transitions include underlying biological predispositions (pharmacogenetic response to nicotine and nicotine metabolism), comorbid

disorders (alcohol dependence, major depressive disorder, and anxiety disorders), and environmental exposures (cigarette taxation, peer smoking, cigarette advertising, antitobacco programs, and parental smoking).

A GWAS, which involves scanning genetic variants across the genomes of many individuals, is among the newest and most powerful methods to uncover unique genes and pathways that contribute to a disorder. These large-scale genetic studies are now possible because of the rapid technological advancements in genetic research. To focus on the examination of genetic factors in the transition from smoking to the development of nicotine dependence, low-level smokers (defined as a lifetime FTND of zero) were compared to nicotine-dependent smokers (defined as having an FTND score of four or more) in a GWAS.[13]

Several novel genes were identified as potential contributors to the development of nicotine dependence in this GWAS, such as neurexin 1 *(NRXN1), TRPC7,* and others. The neurexin genes are expressed in neurons and are hypothesized to influence the balance of excitatory glutamatergic and inhibitory GABAergic synapses.[268] Because substance dependence is modeled as a relative imbalance of excitatory and inhibitory neurotransmission, the neurexin genes are plausible new candidates that contribute to the neurobiology of dependence. An additional piece of evidence on the importance of the neurexin gene family comes from a pooled GWAS by Uhl and colleagues for polysubstance addiction, which identified *NRXN3.*[269] A second gene of interest is *TRPC7,* which encodes a subunit of a multimeric calcium channel. In an animal model using *C. elegans,* genes in this family functionally regulated nicotine-induced neuronal activity.[270] This animal model provides insight into the role this gene may play when nicotine is ingested. Although these results require validation in independent samples, they represent some of the new leads that a GWAS can uncover.

In parallel with the GWAS, a second aim of this genetic project was to examine a comprehensive set of candidate genes to detect variants associated with nicotine dependence. Over 350 genes were genetically queried by using approximately 4,000 SNPs for genotyping. The genes for study included the nicotinic receptors as well as genes known to be involved in the neurobiological pathways that contribute to the development of dependence, such as dopamine and γ-aminobutyric acid (GABA) receptors. Genes were nominated by a skilled committee of investigators from the National Institute on Drug Abuse Genetics Consortium[271] with expertise in the study of nicotine and other substance dependence.

Genetic variants in the nicotinic receptors dominated the association results for nicotine dependence. Genetic association with the *CHRNB3-CHRNA6* nicotinic receptor locus on chromosome 8 was the most significant finding in the candidate gene study, and this cluster was also identified in the GWAS.[13,253] Compelling findings were also seen in the group of SNPs in the *CHRNA5-CHRNA3-CHRNB4* cluster of nicotinic receptor genes on chromosome 15. Evidence shows at least two independent signals in this gene cluster. The first is a genetic variant that codes for a nonsynonymous coding SNP in the α5 nicotinic receptor subunit gene *(*RS16969968).* There is evidence of at least one other independent signal in this gene cluster marked by **RS578776.* These results highlight the importance of the pharmacogenetic response to nicotine as a contributor to the development of nicotine dependence. Tables 2.1 and 2.2 summarize the results from each of the studies. The chromosome 15 findings from

this study subsequently received support from analysis of independent data sets.[272,273] Three studies have further implicated the same gene cluster in predisposition to lung cancer.[274–276] Whether this effect is independent of an effect on smoking is controversial.[277]

In summary, these large-scale studies are a step in the process to identify genetic contributions to nicotine dependence. They can, it is hoped, provide insights to understand the genetic contribution to nicotine dependence so that new approaches can be developed to reduce tobacco use, especially cigarette smoking. Although a substantial majority of smokers report that they want to quit (70%), and an estimated 41% try to quit in a given year, most smokers are not successful (although many are successful over time), and nicotine dependence is a strong predictor of failed

Table 2.1 Results from the Genome-wide Association Study of Nicotine Dependence

SNP	Gene	Chr	Pos(bp)	Risk Allele	Primary *p*-value	Male odds ratio (95% CI)	Female odds ratio (95% CI)
**RS4142041*	*CTNNA3*	10	68,310,957	**G* (0.41/0.34)	5.64E-06	1.7 (1.4–2.2)*	1.1 (1.0–1.4)[a]
**RS999*[b]	*GPSM3, AGPAT1*	6	32,261,864	**C* (0.96/0.94)	1.42E-05	1.9 (1.1–3.5)	2.5 (1.6–4.0)
**RS12623467*	*NRXN1*	2	51,136,740	**C* (0.96/0.92)	1.48E-05	2.4 (1.5–3.9)	1.6 (1.1–2.3)
**RS12380218*	*VPS13A*	9	77,165,214	**G* (0.24/0.19)	2.09E-05	1.2 (0.9–1.6)	1.6 (1.3–1.9)
**RS2673931*	*TRPC7*	5	135,717,335	**T* (0.66/0.61)	3.89E-05	1.7 (1.3–2.1)[a]	1.0 (0.9–1.2)[a]
**RS2791480*	*CLCA1*	1	86,680,605	**G* (0.78/0.72)	4.38E-05	1.5 (1.2–2.0)	1.3 (1.1–1.6)
**RS10490162*	*NRXN1*	2	51,159,308	**T* (0.91/0.86)	5.66E-05	1.9 (1.3–2.8)	1.4 (1.1–1.8)
**RS13277254*	*CHRNB3*	8	42,669,139	**A* (0.81/0.76)	6.54E-05	1.2 (0.9–1.6)	1.6 (1.3–1.9)
**RS10793832*	*FBXL17*	5	107,348,129	**C* (0.32/0.26)	8.13E-05	1.1 (0.9–1.4)	1.5 (1.2–1.8)
**RS2302673*	*FTO*	16	52,625,622	**T* (0.87/0.84)	8.85E-05	1.0 (0.8–1.4)[a]	1.8 (1.3–2.2)[a]

Note. SNP = single nucleotide polymorphism; Chr = chromosome; Pos(bp) = chromosomal position, base pairs; CI = confidence interval. Results for all SNPs are posted at http://zork.wustl.edu/nida. Adapted from Bierut, L. J., P. A. Madden, N. Breslau, E. O. Johnson, D. Hatsukami, O. F. Pomerleau, G. E. Swan, et al. 2007. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Human Molecular Genetics* 16 (1): 24–35.

[a]Significantly different odds ratio for men and women.

[b]The allele frequency for *RS999 is quite different in these data than reported in the SNP database; this may represent a failure to accurately genotype this SNP in this study.

Table 2.2 Results from the Candidate Gene Study of Nicotine Dependence

SNP	Gene	Chr	Pos(bp)	Risk Allele	Primary *p*-value	Male odds ratio (95% CI)	Female odds ratio (95% CI)
**RS6474413*	*CHRNB3*	8	42,670,221	**T* (0.81/0.76)	9.36E-05	1.2 (0.9–1.5)	1.5 (1.3–1.9)
**RS578776*	*CHRNA3*	15	76,675,455	**G* (0.78/0.72)	3.08E-04	1.5 (1.2–1.9)	1.3 (1.1–1.6)
**RS6517442*	*KCNJ6*	21	38,211,816	**C* (0.34/0.28)	5.62E-04	1.4 (1.1–1.7)	1.3 (1.1–1.5)
**RS16969968*	*CHRNA5*	15	76,669,980	**A* (0.38/0.32)	6.42E-04	1.3 (1.1–1.7)	1.3 (1.1–1.5)
**RS3762611*	*GABRA4*	4	46,838,216	**G* (0.93/0.91)	9.22E-04	2.1 (1.4–3.2)	1.3 (0.9–1.8)

Note. SNP = single nucleotide polymorphism; Chr = chromosome; Pos(bp) = chromosomal position, base pairs; CI = confidence interval. Results for all SNPs are posted at http://zork.wustl.edu/nida. Adapted from Saccone, S. F., A. L. Hinrichs, N. L. Saccone, G. A. Chase, K. Konvicka, P. A. Madden, N. Breslau, et al. 2007. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Human Molecular Genetics* 16 (1): 36–49.

smoking cessation. This systematic survey of the genome nominates novel genes that increase an individual's risk of transitioning from smoking to nicotine dependence, and the candidate-gene study has provided persuasive evidence of the role of the nicotinic receptors in the transition from smoking to nicotine dependence. The continued genetic and biological characterization of these genes will help in understanding the underlying causality of nicotine dependence and may provide novel drug development targets for smoking cessation.

Issues in Communication of Genetic Findings

A full discussion of the ethical, legal, and social implications of this research is beyond the scope of this monograph. For a full discussion of these issues, see Caron and colleagues[278] and Shields and colleagues.[279] However, there are several important issues in the interpretation and communication of genetic findings that will be addressed here because: (1) unreplicated findings of gene–nicotine dependence associations could lead to erroneous conclusions based on false-positive results; (2) discrimination or stigma could accrue to individuals or groups identified as being at greater risk for nicotine dependence, especially if the prevalence of genetic risk factors varies as a function of ethnicity or of psychiatric comorbidity; and (3) available genetic tests for nicotine-dependence liability or treatment responsiveness are of questionable value at the individual level. While this portion of the chapter is not intended to be a comprehensive review of all the relevant issues, its purpose is to draw attention to the importance of understanding the broader implications of new research findings and the need for further research and discussion in this area.

The Need to Replicate Gene–Nicotine Dependence Associations

As indicated earlier in this chapter, the bulk of the findings reporting associations between genetic variation and nicotine-dependence phenotypes has been derived from studies of single genes in relatively small samples. The effect sizes tend to be small, and the results have been notoriously difficult to replicate because of cross-study differences in sample ascertainment, population stratification, lack of consistency in SNP genotyping and phenotype definition, and failure to understand the role of linkage disequilibrium. This conclusion applies not only specifically to studies of nicotine dependence but more generally to studies of complex traits.

The advent of GWAS has the potential to alter the course of scientific progress in the field of nicotine dependence, as well as that of many other complex traits.[280] Because it is now possible to study many SNPs (up to 1 million, using the Illumina platform) in multiple genes in relevant pathways, technological advances, if applied carefully, promise to encourage comprehensive investigation of genetic variation in relation to nicotine dependence and to do so in a much more rapid fashion. Concurrent with the application of this new technology are methodological developments centered on the best use of the GWAS approach. Best practices for SNP selection, phenotypic definition, incorporation of prior biological knowledge, multistage genotyping, proper handling of the multiple-comparison problem within the GWAS context, and the critical importance of replication are being proposed and incorporated into requirements for grant funding and publishing in top-tier

journals. Independent confirmation of the association between variation in the α3 and α5 gene cluster on chromosome 15, first reported by Saccone and colleagues,[253] has now been reported by Bierut and colleagues[273] and Berrettini and colleagues.[272]

As of April 2009, the GWAS approach has been successfully applied to two complex traits: age-related macular degeneration and type II diabetes,[281,282] with many more applications of varying maturity being reported in the literature (including atherosclerosis and cancer, both reviewed in Kronenberg;[283] brain aging and cognition;[284] longevity;[285] sleep and circadian phenotypes;[286] and general cognitive ability[287]). It is clear that the GWAS approach will enjoy much popularity in the foreseeable future for the study of complex traits.

However, just as in the previous generation of single-gene, single-variant studies of nicotine dependence, investigators will need to apply with determination and vigilance the fundamental principles of good science and of interpreting results to the lay press and the public. Whereas previous headlines announced, "Gene for smoking identified," the press, now with GWAS results in hand, might broadcast, "Multiple genes for smoking identified." Unless the investigators involved are careful to point out the limitations of their findings and the associated effect sizes (which are destined to be modest with odds ratios of 1.5 or less), it is entirely likely that even more confusion will reign in the public mind as to the meaning of these results.

Discrimination or Stigma May Accrue to Individuals or Groups Identified as Being at Greater Risk for Nicotine Dependence

Several concerns have been raised regarding the potential for information about genetic risk for nicotine dependence or response to smoking cessation treatment based on genotype to be used against individuals or groups in harmful ways.[288] These concerns pose a barrier to physicians' willingness to offer a new genetic test to tailor smoking treatment to their patients[279,289,290] and to smokers' willingness to undergo genetic testing to be matched to optimal treatment. Some of the primary critiques offered with respect to labeling individuals (especially youth) and groups are addressed below.

Might knowledge of one's genetic status with respect to nicotine dependence be useful in deterring potential smokers from initiating smoking? Some might argue that informing adolescents that their genetic profile places them at greater risk of nicotine dependence may give them an incentive not to initiate smoking. However, evidence from other cases indicates that being identified as "at risk" is apt to have little effect on behavior. In the case of phenylketonuria (PKU), for example, dietary management is critical to maintaining phenylalanine levels to avoid developmental problems. In a U.K. study of PKU management, compliance with dietary restrictions to maintain phenylalanine levels among informed children decreased from 70% among children aged 10 to approximately 20% among children aged 15,[291] illustrating the limited ability of personalized feedback about risk to influence adolescents to change their behavior to maintain healthy habits. Thus, it is unclear what, if any, benefit such information might have for smoking prevention in practice.

Several additional risks associated with "labeling" adolescents as being susceptible to nicotine dependence have been identified.[292] Such labeling may result in a sense of fatalism among adolescents, leading to a perceived lack of ability to control their future, a higher willingness to smoke, and a resistance to considering public health messages about the risks of smoking.[293–295] Youths identified as

at higher risk for nicotine dependence who already smoke may interpret this information as meaning that it is futile for them to try quitting. On the other hand, adolescents without a given genetic variant associated with increased risk of nicotine dependence may erroneously believe they can smoke and not become addicted. Thus, the provision of genetic information to adolescents could have significant positive or negative impact and psychological effects. More research is needed to understand adolescents' comprehension of the meaning of genetic risk for nicotine dependence and how this comprehension is likely to affect smoking behavior. The results of this research could then inform proactive public health messages that emphasize the health consequences from tobacco smoke that accrue regardless of genetic background and/or specify tailored methods to reduce chances for nicotine dependence.

Another area of intense debate concerns the framing of genetic information about risk of addiction or response to treatment in racial terms. Despite heated debates and numerous appeals for more careful use of racial categories in genetics research,[296–300] many genetic studies continue to use self-identified racial variables in statistical analyses, resulting in research findings framed in "racial" terms. While it is essential to consider and control for population structure in genetic studies, using self-defined racial or ethnic categories as proxies for human genetic heterogeneity is less scientifically precise (more robust measures are available for assessing geographical ancestry) and fraught with potential for social harm.[299] When research results are framed in racial terms, great harm can accrue to subpopulations identified as more likely to carry certain risk alleles, such as those that confer increased risk of addiction.

A well-documented example of the kind of stigma that can accrue to a particular population is found in the early screening efforts for sickle cell hemoglobin among African Americans, which immediately resulted in considerable racial discrimination in both health insurance and employment contexts. This occurred despite the reality that a similarly high prevalence of the sickle cell trait was found in other subpopulations.[294,301] At the same time, non-African-Americans, who were not socially viewed as being associated with sickle cell, often went undiagnosed until screening was implemented for all newborn infants.

Similarly, research results reporting that genotypes linked to nicotine dependence, cocaine, and other substances occur at a higher rate in African Americans than in European Americans holds the potential for exacerbating existing racial discrimination. Such research results are not received in a vacuum but are read in the context of social history and can lead to racism and marginalization of an entire portion of society, given the contentious history of racial stereotypes in the United States.[301] Studies have shown, for instance, that physicians already prescribe pain medication in smaller doses to African American patients than to European American patients with similar symptoms, reflecting a possible assumption that African Americans are more likely to become addicted to opiates.[302,303] Because of the well-documented racial disparities in access to and quality of health care,[304–309] investigators must seriously consider the unintended consequences of incorporating genetic information into risk assessment related to nicotine dependence.

The Association between Gene Variants, Nicotine Dependence, and Psychiatric Conditions May Also Result in Increased Risk for Stigmatization

As has been argued by Shields and colleagues, social sensitivity related to

the pleiotropic associations of genetic variants implicated in nicotine dependence or response to treatment are intensified when they intersect with data on racial differences in the frequency of such risk alleles.[299] One feature of the genetics of complex traits, such as smoking, that raises a host of social and ethical concerns is the pleiotropic associations of key genetic variants with many other traits. An early example of a pleiotropic genetic test is the test for apolipoprotein E, which simultaneously provides information on risk for cardiac disease and risk of developing late-onset Alzheimer's disease.[310–312] Genes hypothesized to play a key role in increased risk of nicotine dependence also have been associated with increased risk of addiction to cocaine, alcohol,[313,314] sexual activity,[315] compulsive gambling,[313] novelty seeking,[316,317] and to other neuropsychiatric conditions. (Table 2.3 by Shields and colleagues,[299] Billett and colleagues,[318] Comings and colleagues,[319,320] Muglia and colleagues,[321] Nielsen and colleagues,[322] and Rowe and colleagues.[323]) Many of these conditions and behaviors are very socially sensitive.[324–329] Persons identified as having these genotypes may be stigmatized or discriminated against. One might assume that persons finding out they had a genetic profile of increased risk for nicotine dependence if they experimented with cigarettes might be deterred from initiating smoking. However, this profile could not be obtained without simultaneously generating information with other, more onerous implications. Similarly, it might be useful to tailor smoking cessation treatment to genotype to match patients to the treatment likely to work best for them (see below for a discussion of the evidence for the use of such tests), but such genetic testing would simultaneously generate additional information about a person's genetic risk for other addictions and psychiatric conditions. For these reasons, Shields and colleagues have argued that, in weighing the pros and cons associated with decisions regarding genetic testing to tailor smoking prevention

Table 2.3 Pleiotropic Associations of Genetic Variants Implicated in Smoking

	Complex Traits			
Genetic Variants	**Tobacco Use**	**Addictive Behaviors**	**Psychiatric Conditions**	**Behavior Patterns**
Dopamine Pathway				
DRD1 (dopamine D1 receptor)	Smoking	Cocaine, Alcohol	Tourette's Syndrome	Gambling
DRD2 (dopamine D2 receptor)	Smoking	Alcohol, Cocaine	ADHD,[a] PTSD[b]	Sexual Activity
DRD4 (dopamine D4 receptor)	Smoking	Alcohol	ADHD,[a] OCD[c]	Novelty seeking
SLC6A3 (dopamine transporter, DAT)	Smoking	Alcohol	Anxiety, Tourette's Syndrome	
DBH (dopamine beta-hydroxylase)	Smoking		Paranoia	
Serotonin Pathway				
5HTTLPR (serotonin transporter)	Smoking	Alcohol	Depression, Anxiety	
TPH (tryptophan hydroxylase)	Smoking	Alcohol	Suicide, Depression	Aggression

[a]ADHD = attention deficit hyperactivity disorder

[b]PTSD = post traumatic stress disorder

[c]OCD = obsessive compulsive disorder

and treatment strategies, decisions should be made based on the most potentially harmful uses of information generated by such testing.[288]

In what ways might these pleiotropic associations exacerbate concerns about identifying individuals at increased risk for nicotine dependence or raise new concerns? There have been cases of insurers increasing premiums or denying coverage to beneficiaries on the basis of genetic susceptibility tests for breast and ovarian cancer and for Alzheimer's disease.[330] Smokers have long been charged higher health insurance premiums and identified as a socially stigmatized group.[331] In addition, the well-established adverse impact of smoking on employers' health care costs and worker productivity has led to instances in which employers have discriminated against smokers in hiring practice.[332] It is therefore not impossible to imagine that some employers might consider genetic testing as a screening tool in considering prospective employees. Such discrimination would likely be exacerbated when this genetic status is linked to an increased risk of alcohol or drug addiction, since this is a source of high health care costs.[333] Such discrimination might be more likely to take place within self-insured firms, in particular, since they more directly manage and bear the costs of their employees' health care.[334]

The issue of harm to individuals from disclosure of genetic information is not new, and this issue has been addressed in many contexts.[335–338] While some progress was made in protecting individuals against discrimination with the 1990 Americans with Disabilities Act (ADA)[339] and Executive Order 13145, which prohibits discrimination against federal employees on the basis of genetic information,[340] no comprehensive federal law bans genetic discrimination for the general population. State laws remain the primary source for protection of genetic information. As of 2007, only 41 states banned genetic discrimination by health insurance companies, and only 32 states had passed laws that ban the misuse of genetic information by employers.[341] Greater federal protections are provided by the passage of the Genetic Information Nondiscrimination Act (GINA) of 2008.[342] On April 24, 2008, the Senate amended and passed GINA as H.R. 493. The House reconciled and agreed to the Senate bill on May 1, 2008.

Although GINA became law under President George W. Bush and addresses many concerns about discrimination and privacy, gaps remain in protection, including important omissions in consumer protections against employers discriminating against potential employees on the basis of genetic status.[343,344] As Rothstein points out, GINA makes it unlawful for an employer to request, require, or purchase genetic information about an employee or applicant, yet section 102(d)(3) of the ADA still allows employers to require a signed authorization to release all of an individual's health record (including genetic information) after a conditional offer of employment.[344] Moving forward, it will be essential to identify and close persisting gaps in protections to reassure patients who may benefit from genetic testing that information from such tests will not be used to discriminate against them in health insurance or employment. Failure to address these gaps will seriously undermine any future efforts to use genetic information to guide smoking prevention or treatment strategies.

The research involving genetics and nicotine dependence (and associated concerns) is occurring within the context of a much broader series of developments at the federal level, as described in the document, "Personalized Health Care: Opportunities, Pathways, Resources."[345] The report identifies several future outcomes of personalized health care:

(1) prediction of individual susceptibility to disease, (2) provision of more useful and person-specific tools for preventing disease, (3) detection of the onset of disease at the earliest possible moment, (4) preemption of the progression of disease, and (5) targeting of medicines and dosages more precisely and safely to individual patients (p. 1). In addition, the report identifies the need to (1) make the individual patient's health information available on demand, (2) provide necessary support to clinicians when needed to use information concerning genetic and molecular factors, (3) bring large data sets together from real-world medical practices through secure networks to accelerate identification of best and safest practices, and (4) use data from data networks to understand differences in patients' responses to drugs and other therapies (p. 2).

The Value of Genetic Tests to Assess for Nicotine-Dependence Liability or Treatment Responsiveness Is Questionable

Despite the best efforts and intentions of the scientists involved in the work discussed in this monograph, vigilance and proactive planning are needed to minimize the risk of misunderstanding, misinterpreting, misusing, or otherwise abusing the results demonstrating associations between genetic factors and nicotine dependence.[278] Documented examples exist of at least some instances of unintended consequences of this work. For example, a commercial company has been created to promote the sales of a genetic test (in this case, *DRD2*) that purports to predict the likelihood for success (smoking cessation) in response to certain pharmacological agents. Not only is there an inadequate knowledge base to support the widespread clinical use of this test, but also the cost-effectiveness of such a test has been called into question.[346,347]

More generally, the rapidly developing field of direct-to-consumer marketing of genetic tests with little or no supporting evidence of their value at the individual level has generated a great deal of concern in the literature[348] and calls for a regulatory framework to protect consumers from misleading claims made by commercial interests promoting these tests.[349] Scientists in the field of genetics and nicotine dependence will need to stay informed regarding developments in this area of personalized medicine so that their work can be placed in the broader context of this emerging field.

The majority of scientists involved in the work described in this monograph are most interested in the implications of their work for understanding basic processes underlying nicotine dependence and, more generally, addiction. They are far less, if at all, interested in turning this work into for-profit, commercially available tests or products. Nevertheless, the ethical scientific community must be vigilant to quickly identify and challenge claims made about a test's predictive value for assigning smoking cessation treatments at the individual level. Similar concerns arise for claims that genetic variation can be used to predict whether a young child will become addicted to tobacco despite the fact that scientific work in this area has only just begun to explore this question.

Simply put, the work described in this monograph and in the field of genetics and nicotine dependence is in an early stage, and the body of available evidence is not sufficient to support any kind of predictive testing at the individual level. This may not be the case in other fields, such as the genetics of cancer, in which many decades have been spent by thousands of scientists to identify the genetic basis of cancer. By comparison, the field of genetics and nicotine dependence represents a tiny fraction of the total effort in the

field of cancer genetics, even though tobacco use remains an undisputed major risk factor for cancer. Evidence of a potential overlap between gene variants in the α3–α5 nicotinic receptor cluster on chromosome 15, which are associated with nicotine dependence[13,253,272] and with lung cancer,[274–276] however, may cause the two fields to converge.

Summary

This chapter provides a framework for understanding nicotine-dependence phenotypes and an overview of major concepts, along with a summary of selected findings from the tobacco genetic literature. This chapter also raises important issues as to how genetic research is communicated and understood by the media and the public.

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