1

Overview and Conclusions

This chapter introduces a monograph that examines the relationship between genetics and nicotine dependence. It summarizes evidence and research accumulated since the 1950s on the effect of both unmeasured and measured genetic factors, as well as that of behavioral and environmental phenotypes on nicotine dependence.

This chapter frames issues addressed in the monograph, and describes its organization around topic areas including relating genetic and gene-environment factors to tobacco use, linking genetic traits with measures of nicotine dependence, examining the progression of tobacco use from adolescence to adulthood and its potential relationship to other substance abuse, identifying genetic liability markers for nicotine dependence in chronic smokers, and exploring the future of genetic studies for nicotine dependence. In addition to noting several "firsts" accomplished with the completion of this monograph, the closing sections of this chapter present volume and chapter conclusions generated by the work presented here.

Experts in psychology, psychiatry, behavioral pharmacology, neurobiology, epidemiology, child development, statistical genetics, and bioinformatics were assembled to provide data analyses within these pages. It is hoped that this monograph will help define various groups of smokers to advance the field of behavioral genetics of nicotine dependence.

Introduction

Substantial evidence, accumulated since the 1950s, from the study of twins, siblings, and nuclear families shows that *unmeasured* genetic factors (influences estimated from analyses of correlations among family members for specific phenotypes) influence the likelihood of both initiating and maintaining nicotine dependence. Beginning with research published in 1994, studies show that *measured* genetic factors (influences estimated from analyses of associations between genomic regions or specific gene variants and specific phenotypes) and nicotine dependence are also related. More than 100 published papers have reported associations between tobacco use behaviors and variants of candidate genes or genomic regions in relevant neurobiological and metabolic pathways. The combined evidence reveals that genetic involvement in nicotine dependence is present in adolescents and adults, both males and females, and in several cultures.

Concurrent with the work started in the early 1990s, new genomic technologies were introduced that make previous research quickly obsolete. With the rapid decrease in costs to genotype individuals for very large numbers of variants across the whole genome, the whole-genome association study has now become possible. Similarly, it is now possible to genotype candidate genes for many variants (known as single nucleotide polymorphisms [SNPs]). SNPs account for a large number of functional variants in humans, which has made them particularly useful in whole-genome research. Along with genomic technology, advanced methods from the experimental, bioinformatic, statistical, and epidemiological fronts now make it possible to envision the next generation of studies. These advances will support the further integration of neurobiological mechanisms into public health efforts.

The marked decline in cigarette consumption in the United States since the 1960s corresponds to increased public awareness of the dangers of tobacco use, changing social norms about tobacco, and increasing 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 the 1980s, smoking was present in most public places, with smoking allowed virtually everywhere (except in areas of increased probability of fires or damage 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, tobacco use screening and brief intervention by clinicians has become a top-ranked clinical preventive service on the basis of health impact, effectiveness, and cost-efficiency, further reducing cigarette consumption.

Despite enormous progress in the public health arena, 45 million individuals remain regular users of tobacco, with an estimated annual cost to the U.S. economy of $167 billion due to premature death and disability.[1] Worldwide, approximately 1 billion people are regular users of tobacco, and 3–6 million people die every year from illnesses caused by tobacco.[2] One reason for this is that broad public health and community efforts, though found to be effective by such groups as the U.S. Task Force on Community Preventive Services, are not widely implemented and have varied substantially across the United States. Also, tobacco settlement dollars are spent by many states on non-health-related and non-tobacco-related activities instead of tobacco control and prevention.[3]

Another reason for continued smoking is that the potential of powerful genomic

tools to answer important questions about nicotine dependence has yet to be maximized, and increased attention should be paid to the nature of the behavioral and/or environmental phenotypes included in future genomic studies. The majority of the published work has relied upon relatively broad, nonspecific measures of nicotine dependence that may, in fact, represent the end result of a series of initiating, promoting, and maintaining factors, many of which interact along the developmental pathway to result in full-blown adolescent and adult nicotine dependence. The preparation of this volume was undertaken to provide future genomic investigations of nicotine dependence with a review of more refined phenotypes that derive from theory-driven and/or experimental work. To accomplish this, a team of experts in the areas of psychology, psychiatry, behavioral pharmacology, neurobiology, epidemiology, child development, statistical genetics, epidemiology, and bioinformatics was assembled to review the available evidence for novel phenotypes that could, in turn, meet the requirements of an "endophenotype."

Endophenotypes are presumed to be more directly related to the underlying characteristics of nicotine dependence than are broad inclusive measures. To be viewed as an endophenotype, a candidate phenotype may be neuropsychological, neurophysiological, neurobehavioral, biochemical, endocrinological, or neuroanatomical in nature and must be heritable, state-independent, cosegregate with nicotine dependence in families, and present at a higher rate among unaffected relatives of those with nicotine dependence than in the general population.[4,5] One of the assumptions of the endophenotype concept is that these constituents will have simpler genetic underpinnings than does nicotine dependence itself. The use of endophenotypes in genetic research of nicotine dependence has been underutilized. Another underutilized approach to the study of nicotine dependence involves the study of gene-environment interactions in which it is assumed that "environmental pathogens" cause the expression of a disorder such as nicotine dependence only in the presence of certain gene variants.[6]

One of the objectives of the present volume is to more fully explore the existing approaches and supporting evidence (at both the phenotypic and environmental levels) available to the next generation of genetic studies. These approaches include factors and processes that are tobacco specific, as well as those that are related to broader correlated conditions, including other forms of substance dependence.

About the Monograph

The overarching goal for the volume was for each of the contributing editors and authors to review the existing literature, and to go beyond it, by identifying new concepts, measurements, and strategies to more fully enrich the universe of discourse and investigation in the area of genetics and nicotine dependence. The authors were asked to summarize the best available evidence and make recommendations accordingly. In some cases, when the available evidence is thin or nonexistent, the authors were asked to conduct original analyses or apply innovative methods to existing data to move the field forward. In addition, the authors were asked to provide informed opinions as to where the next generation of research should head.

The monograph will be most useful to individuals who are or will be designing next-generation studies to determine causal relationships between genes and nicotine dependence. To limit the scope of the volume, the emerging literature on the relationship between genetic variation

and response to pharmacotherapy for smoking cessation is not included. While the volume will identify many important issues and questions concerning nicotine dependence and its measurement, it does not seek to resolve the issue of what nicotine dependence is nor does it seek to identify the "best" measures of nicotine dependence. Throughout the volume, the reader will clearly see where the evidence is solid in support of a phenotype's potential role as an endophenotype and where the evidence is weak or simply not yet available.

The chapters of the monograph are organized into six parts.

Part 1—Overview, provides background context for research in genetic and gene-environment factors in tobacco use. It focuses on phenotypes and endophenotypes that may link genes and behavior and be a basis for future genetic studies. In addition, conceptual, theoretical, and methodological considerations in the further study of nicotine dependence are examined.

Part 2—Theoretical Considerations, examines the theoretical basis for constructs that may link heritable genetic traits with observable measures of nicotine dependence. These include phenotypes representing a causal path between specific genetic actions and measures of nicotine dependence, as well as endophenotypes measuring indirect influences, such as those found before nicotine exposure. This part examines theoretical issues in establishing nicotine-dependence phenotypes as well as studies of human and animal behavior.

Part 3—Developmental Trajectories of Tobacco Use and Their Relation to Tobacco Dependence, examines issues in the study of trajectories of tobacco use and their future potential as a basis for genetic studies of nicotine dependence. Chapters include a literature review of developmental trajectories of cigarette smoking between adolescence and adulthood, genetic modeling issues in the study of smoking trajectories and behavior, and the relationship of these with other trajectories such as alcohol use or substance abuse.

Part 4—Endophenotypes. Endophenotypes serve as intermediary measures that have the potential to provide a link between genes, smoking behaviors, and nicotine dependence. Endophenotypes may help serve as a basis for future studies to identify genetic liability markers for nicotine dependence. This part discusses the evidence base for several candidate endophenotypes for nicotine dependence at or before initial exposure to nicotine as well as for endophenotypes for nicotine dependence in chronic smokers.

Part 5—Epidemiological and Methodological Considerations, examines epidemiological and methodological issues related to the future of genetic studies of nicotine dependence. These issues include the use of epidemiologically-based phenotypes for tobacco use; a potential etiological architecture for genetic and environmental influences on smoking phenotypes; and the hierarchical modeling of gene-gene joint action.

Part 6—Future Directions, comments on how continued research in the area of genetics may influence future understanding of the pathways responsible for nicotine dependence, the role genetic variation plays in its initial acquisition and maintenance. It also provides summaries and recommendations from each of the parts of the monograph and concludes with several cross-cutting suggestions for future work in this area.

A Note to the Reader

It is not the intention of the authors of the volume to suggest that the continued substantial prevalence of nicotine dependence in the population is solely

determined by genetic factors. Much of the early work in twins indicates that environmental influences are equally important (and may be more so at different phases of the development of nicotine dependence). An enormous 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 its use. Protobacco stimuli are ubiquitous in the environment and include advertising by the tobacco industry and the portrayal of smoking in the movies. Equally important, the tobacco industry controls the design of cigarettes and, so, the bioavailability of nicotine. The form in which the nicotine is delivered is an important variable that almost certainly interacts with the biological factors discussed in the report.

No published study has adequately addressed simultaneously genetic variation, quantitative measures of social and cultural variation, and the interaction between the two sources of variation. This gap reflects the fact that scientists from the two traditions have not typically worked with each other rather than a dismissal of each other's work. Tobacco use as reflected in population trends is the product of the interaction of agent, host, and environmental factors. Government policies are important modifiable environmental influences that can alter how tobacco products are designed and marketed (agent factors) and how consumers respond. Individual variation in host responses to tobacco is important to understand, since this has implications for understanding how different people will respond to different programs and policies (i.e., treatments for nicotine dependence, tax increases, mass media campaigns, etc.). Synergy occurs when tobacco control and prevention interventions directed at agent, host, and environmental factors are implemented together.

Major Accomplishments

In completing the volume, several "firsts" were accomplished:

- The first comprehensive review of the state-of-the-art in the measurement of nicotine dependence and related phenotypes within the context of genetic studies
- The first demonstration that heterogeneity in tobacco use trajectory from early adolescence to early midlife is related to both family history of smoking and to nicotine dependence in adulthood
- The first demonstration that conjoint trajectories of tobacco and alcohol use in adolescents are heritable
- The first review of biobehavioral phenotypes that could be utilized by future genomic studies of pre- and postnicotine exposure
- The first demonstration that microcontextual effects on nicotine dependence can be assessed and are informative within the context of a genetically informed study
- The first use of Bayesian analysis as informed by a nicotine metabolic ontology to determine the relative importance of several genes to variation in nicotine metabolism

Major Conclusions

Several broad conclusions emerge from the volume. These include

1. At every level of analysis (theoretical, animal, child, and adult), good candidate endophenotypes are available for inclusion in future genomic studies of nicotine dependence.

2. Results from the animal and human domains implicate the importance of nicotinic acetylcholine receptors in nicotine self-administration, reward, and dependence.
3. Developmentally, there is evidence from latent class growth analysis and growth mixture modeling in unrelated and related adolescents that familial and/or genetic factors play a role in trajectories of tobacco use that vary in age of onset, level, and chronicity of use, as well as in the extent to which tobacco and alcohol use co-occur.
4. In children and adults, there are neuropsychological, electrophysiological, and behavioral laboratory measures characterized in other research contexts that may shed light on mechanisms that promote risk for initiation and maintenance of nicotine dependence.
5. Along with more refined definitions of nicotine dependence at the epidemiological level and an increased number of options at the phenotypic level, several technological developments will be important to the next generation of studies of nicotine dependence, such as whole-genome genotyping, epigenetics, proteomics, and metabolomics. Complementing these technologies are methodological advances including Bayesian statistics, behavioral ontologies, identification of developmental trajectories, and real-time measurement of environmental antecedents to nicotine dependence.

Chapter Summaries and Conclusions

Part 1—Overview

Chapter 1. Overview and Conclusions

Chapter 1 provides an introduction and framework for the monograph, describes how it is organized, and includes major volume conclusions, chapter summaries and conclusions, and a look to the future.

Chapter 2. Genetic Studies of Nicotine Dependence: Current Status

Chapter 2 begins with a brief summary of the epidemiology of tobacco use, focusing on environmental factors that have been shown to promote and reduce smoking behavior. Also presented is an integrative model of tobacco use and nicotine dependence, illustrating the concept of trajectories of phenotypic pathways. The chapter then provides a history of research in the genetic basis of nicotine dependence. A full discussion of the limitations in the conceptual understanding of the construct of nicotine dependence along with a detailed framework for moving the field forward is then presented. Next follows a summary of findings from selected (biometric and measured) genetic studies of nicotine dependence. The chapter ends with a brief discussion of some of the major issues in communicating genetic findings.

Part 2—Theoretical Considerations

Chapter 3. The Nicotine-Dependence Phenotype: Translating Theoretical Perspectives and Extant Data into Recommendations for Genetic Mapping

Chapter 3 examines theoretical issues in establishing nicotine-dependence phenotypes, including distal measures of nicotine dependence focusing on mature nicotine dependence, newer multidimensional measures of nicotine dependence that examine motivational factors leading to dependence, and endophenotypes and transitional phenotypes that may form a causal path

between specific genetic actions and measures of nicotine dependence.

Conclusions

1. Most widely used tests of nicotine dependence, such as the Fagerström Test for Nicotine Dependence and the *Diagnostic and Statistical Manual of Mental Disorders,* aggregate data across different dimensions of dependence, thereby compromising the reliability and validity of these measures. Evidence suggests, however, that selected items from these measures and from newly developed dependence scales can be relatively coherent, show fairly high heritability, and be consistently related to core dependence features such as relapse likelihood.

2. Although key variance associated with the dependence construct will be captured by measures of smoking rate, latency to smoke in the morning, and the likelihood or latency of relapse, other complementary measures should also be considered such as strength of withdrawal symptoms and perceived control over smoking. Analytic strategies should adjust for environmental factors such as home or work smoking restrictions, which, in theory, may reciprocally affect dependence itself.

3. Nicotine dependence involves both environmental and constitutional influences, and the effects of genetic variants associated with nicotine dependence require certain environmental conditions to influence the phenotype (at minimum, drug access and use). Determining which environmental features moderate genetic expression and how to incorporate such gene-environment interactions into genetic mapping remains an area for further study.

4. New developments in the assessment of the nicotine-dependence phenotype include the development of new multidimensional measures of nicotine dependence, including the Nicotine Dependence Syndrome Scale and the Wisconsin Inventory of Smoking Dependence Motives. These measures of mature dependence phenotypes provide the opportunity to measure relatively discrete dimensions of dependence and may permit more specific gene mapping.

5. In addition to greater specificity, it is vital to capture important developmental processes that may be masked by the mature nicotine-dependence phenotype. To obtain measures sensitive to particular biological mechanisms that may have close links to genetic variants, researchers may need to develop biological, behavioral, and cognitive neuroscience assays that complement self-report measures. These may include measures of endophenotypes, or intermediate phenotypes, that assess vulnerabilities to dependence that preexist nicotine use as well as transitional phenotypic measures that assess processes that change in response to drug exposure and that lead to mature dependence.

6. All stages of the genetic mapping of nicotine dependence should be guided by specific theory linking candidate genetic variants sequentially with critical biological and behavioral processes and, ultimately, with phenotypes of clinical significance.

Chapter 4. Mouse Models and the Genetics of Nicotine Dependence

Chapter 4 examines key issues in using mouse models for nicotine dependence, including how nicotinic acetylcholine receptors contribute to tissue-specific response within the context of strain-specific genetic background, the interaction of nicotine with physiological systems and

how experimental results with mice may relate to the physiology of human smoking, and the way mouse models recapitulate many basic features of nicotine dependence in humans.

Conclusions

1. Substantial differences exist between mouse strains in their response to the acute or chronic administration of nicotine. These differences implicate specific neuronal nicotinic acetylcholine receptors within a broader genetic context, which suggests a central role for these genetic variants in nicotine dependence in humans.
2. The three most common routes of administration (intravenous, subcutaneous, and oral) for nicotine in rodents vary in the degree to which they model key features of human nicotine dependence, such as the behavioral features of self-administration and the acute and chronic physiological effects of nicotine. Each administration route offers advantages and disadvantages. Intravenous self-administration permits self-administration but may entail receptor-level response artifacts due to high dosages. Subcutaneous administration allows experimenter control of dosage and withdrawal over long time periods at a cost of precluding self-administration. Oral administration via drinking water permits chronic nicotine exposure and produces evidence of dependence, but is subject to specific possible side effects, making this issue an important variable in research design.
3. While mice generally are less sensitive to nicotine than are rats, mouse models now have a strong research base for nicotine effects. Mice are amenable to genetic and pharmacological experimental manipulation. They exhibit heterogeneity in strain-specific responses to nicotine, and methods of homologous recombination permit manipulation of specific genes. Data now link specific mouse strains to genetically influenced differences in the effects of nicotine exposure that can facilitate further study of nicotinic acetylcholine receptor biology in mice.
4. Mouse models link nicotine self-administration to high-affinity nicotinic acetylcholine receptors, genetic differences, developmental factors, and other potential mechanisms of dependence. These models have, in addition, linked nicotine reward in the form of conditioned place preference with genetic strain differences and specific receptor subtypes and have linked acute and chronic nicotine tolerance with other genetic and receptor differences. The models have also linked the $\alpha 7$ and $\alpha 4\beta 2$ receptors with nicotine enhancement of working memory, learning, and attention and have shown strain-specific aging effects on nicotinic acetylcholine receptor expression.
5. Although substantial differences exist in the biology of nicotinic acetylcholine receptor expression and function between mice, other rodents, and humans, nascent research in mouse models for nicotine dependence shows considerable promise in furthering understanding of the biology and genetics of nicotine dependence.

Part 3—Developmental Trajectories of Tobacco Use and Their Relation to Tobacco Dependence

Chapter 5. Developmental Trajectories of Cigarette Smoking from Adolescence to Adulthood

Chapter 5 examines literature concerning developmental trajectories of cigarette smoking between adolescence and

adulthood and presents an empirical example of these trajectories. This chapter also provides a framework for part 3 that explores aspects of cigarette smoking trajectories and their potential to inform further genetic research.

Conclusions

1. Previous studies (and the empirical example presented in the chapter) have identified multiple developmental trajectories of tobacco use from adolescence to adulthood. These trajectory groups, which vary in age of onset, rate of acceleration, and persistence of smoking over time also vary in their antecedents and correlated risk factors. These trajectories may be informative as developmental phenotypes for genetic studies of tobacco use.
2. Statistical approaches such as latent class growth analysis and growth mixture modeling can be useful in evaluating developmental trajectories of smoking behavior. However, challenges in using these approaches include the handling of within-class random effects, the impact of a nonnormal aggregate distribution on the classes extracted, the need for proper model specification and parameterization, the span of evaluated data, and the impact of abstainers on the model.
3. Analysis of a 25-year cohort-sequential study of smoking behavior identified six distinct trajectories of smokers across eight waves of data collection. These trajectory groups were experimenters; developmentally limited smokers; early-onset, persistent smokers; high-school-onset, persistent smokers; late-onset, persistent smokers; and successful quitters, with a priori groups of stable abstainers, stable quitters, and relapsing/remitters. Trajectory group membership was related to educational attainment, family history of smoking, and indicators of nicotine dependence.

Chapter 6. Genetic Modeling of Tobacco Use Behavior and Trajectories

Chapter 6 examines genetic modeling issues in the study of smoking trajectories and behavior, including methodological and conceptual issues, statistical modeling considerations, a review of prior genetic studies of smoking behavior, and a study applying an item response theory approach to an analysis of smoking trajectories.

Conclusions

1. Data from twin studies suggest that shared environmental factors are the predominant source of familial resemblance in liability to smoking initiation in young adolescents, while additive genetic factors appear more important in older adolescents.
2. Results from extended twin designs show that significant assortative mating exists for smoking initiation and that the parent-child correlations can be almost entirely accounted for by genetic factors. This implies a limited environmental influence of parental smoking initiation on smoking initiation in their children.
3. In contrast to the significant role of shared environmental factors in smoking initiation, the liability to smoking persistence and nicotine dependence appears to be primarily accounted for by additive genetic factors. Furthermore, the liabilities to initiation and progression appear to be substantially correlated. Molecular genetic studies may be expected to find some genetic variants that contribute specifically to initiation—some that are specific to dependence and some that contribute to both.
4. Future development and applications of genetic latent growth curve models and genetic latent class models promise to improve the understanding of the role

of genes and environment in smoking trajectories and transitions from nonsmoker to smoking dependence.

5. The search for susceptibility loci for smoking-related traits, either through linkage or association studies, has not identified any convincing replicated findings. However, several genomic regions and several candidate genes have been found to be associated with smoking behavior in more than one study.

6. Improving the assessment of nicotine initiation and dependence by allowing for differences in measurement by age and gender and taking conditionality into account might provide more accurate estimates of the contributions of genes and environment to different stages of smoking.

7. Meta-analyses or mega-analyses of studies of smoking phenotypes—both genetic epidemiological and molecular genetic—should prove useful in summarizing the available data and results. Possibly, certain data sets may produce results that are outliers, and controlling for their effects would permit finer resolution between hypotheses and more accurate parameter estimates.

Chapter 7. Trajectories of Tobacco Use from Adolescence to Adulthood: Are the Most Informative Phenotypes Tobacco Specific?

Chapter 7 examines the evidence base for linkages between substance-use trajectories, as well as the results of an original empirical study examining smoking and alcohol use over time across a cohort group of male twins. The areas discussed include common versus specific liability to substance-use disorders, covariate relationships between smoking and other substance-abuse trajectories, and conjoint trajectories of smoking and other substances.

Conclusions

1. Studies examining the developmental course of multiple substances have shown relatively high concordance between identified trajectories despite diverse course shapes and different course prevalences.

2. Membership in a given developmental trajectory, which can be captured by a single categorical latent variable, represents age of onset and severity as well as change (slope) in use of a substance; moreover, membership in a trajectory characterized by concurrent use of two (or more) substances simultaneously provides information for multiple substances.

3. Developmental course might serve as a valuable phenotype for biometric models, and determining the degree to which a phenotype of developmental course is substance specific is valuable for the genetic study of addictive behavior.

4. Evidence using twin data indicates that courses of substance use are genetically influenced, with monozygotic twins showing greater concordance for smoking and for drinking than do dizygotic twins. The genetic contribution to the risk of taking different pathways in development represents an area for further study.

5. Conjoint trajectories of drinking and smoking reveal even greater concordance than do single-substance trajectories, suggesting greater heritability for courses extracted from several substances. This underscores the value of considering substance use across multiple domains when constructing phenotypes for research and perhaps even for clinical use. However, extending the concept of the components of developmental substance-use phenotypes raises new questions such as, Which substances? What aspects of substance use or its consequences? Which periods

of development? Thus, the findings show the value of extending the concept of substance-use phenotypes but not necessarily optimal phenotypes that "carve nature at its joints."

6. If resources are limited for genetic analyses, focusing on those with the most "extreme" phenotypes marked by both high initial level and chronic continued use may represent an efficient strategy for identifying genes associated with more problematic forms of substance use.

Part 4—Endophenotypes

Chapter 8. Endophenotypes for Nicotine-Dependence Risk at or before Initial Nicotine Exposure

Chapter 8 examines the evidence base for several candidate endophenotypes for nicotine-dependence risk at or before smoking and nicotine exposure. Issues covered include approach-related smoking risk variables, avoidance-related smoking risk variables, control-related smoking risk based on psychological variables, and measures of initial response to nicotine exposure.

Conclusions

1. Several higher-order psychological constructs can consolidate many smoking initiation and progression risk variables. These constructs, as well as sensitivity to initial nicotine exposure, can be related to observable neural, physiological, and behavioral measures that may, in turn, serve as potential candidate endophenotypes for genetic research on nicotine dependence.
2. Several laboratory measures exist that could be associated with the risk for smoking initiation and progression and subsequent nicotine dependence, but these associations have yet to be investigated. Findings are mixed for the reliability and heritability of these measures, and minimal evidence exists for their validity, representing an area for further study.
3. Measurement of sensitivity to initial nicotine exposure is subject to numerous methodological limitations, including ethical difficulties with empirical measurement in naive (e.g., previously unexposed to nicotine) subjects, a lack of consideration of smoking dose and context from retrospective self-reports, recall bias, and self-selection to early smoking experience. At the same time, preliminary findings indicate that measures of reward and mood effects surrounding initial exposure to smoking show promise as a potential basis for endophenotypes of a genetic predisposition to nicotine dependence.
4. The available evidence points to the plausibility of endophenotypes that link factors at or before initial nicotine exposure with the potential for nicotine dependence. These endophenotypes reflect approach, avoidance, and control-related traits as well as initial sensitivity and exposure measures in response to nicotine intake. Further research is needed to help identify endophenotypes that connect risk variables for nicotine dependence to genetic influences.

Chapter 9. Nicotine-Dependence Endophenotypes in Chronic Smokers

Chapter 9 explores the evidence base for purported endophenotypes for nicotine dependence in chronic smokers. Motivational measures, sensory measures, measures of cognitive function, measures of abstinence-induced and cue-induced craving, and affective regulation and impulse control are discussed from a standpoint of biological plausibility, objective measurement criteria and

reliability, genetic influences, and association with nicotine dependence.

Conclusions

1. Nicotine dependence in chronic smokers is characterized by persistent smoking behavior despite knowledge of its harm (e.g., an inability to sustain a quit attempt). Reinforcement measures such as nicotine choice have been related to nicotine dependence, although further research is needed on the relationship between dependence and ad libitum drug self-administration, behavioral choice, and progressive ratio measures. Genetic studies in reinforcement measures in mice indicate a potential for studying the heritability and genetic influence for these behaviors in humans.
2. Limited evidence exists regarding the relation between self-reported measures of reward and nicotine dependence in humans, while animal studies show a potential link between the reward-related measure of conditioned place preference and nicotine dependence.
3. Evidence of heritability and genetic influence has been established for measures of sensory processing, such as resting electroencephalogram activity, event-related potentials, and the prepulse inhibition of startle response, as well as cognitive measures such as attention and working memory. Further research is indicated to investigate the relationship of such measures to nicotine dependence in humans.
4. Self-report measures of abstinence-induced craving have been related to the success of cessation efforts (i.e., dependence), while neither cue-related craving nor psychophysiological measures of craving have been reliably shown to relate to nicotine dependence. The relationship of these measures with genetic factors remains an area for further investigation.
5. Self-reported levels of negative affect following smoking cessation have been strongly related to smoking persistence. Persistence has also been associated with abstinence-induced changes in physiological measures such as cortisol and the dehydroepiandrosterone to cortisol ratio. Other measures of affect have not been shown conclusively to relate to measures of nicotine dependence.
6. Impulsivity and cognitive control measures such as delay discounting, the go/no-go task, and the Stroop interference task have not been shown conclusively to relate to nicotine dependence, while the go/no-go task has shown some evidence of heritability and relation to genetic factors.
7. Overall, the available evidence supports the possibility of endophenotypes for nicotine dependence in chronic smokers on the basis of motivational factors and, to a lesser extent, sensory, cognitive, affective, and behavioral measures. Further research is indicated to help establish a consistent pattern of heritability, genetic influence, and association with nicotine dependence for measures in each of these areas.

Part 5—Epidemiological and Methodological Considerations

Chapter 10. Epidemiological Analysis of Variation in Phenotypic Definitions: A Proof of Concept Using an Example of a Cessation Phenotype

Chapter 10 explores the use of an epidemiological approach for modeling smoking phenotypes that are based on transitions along the smoking trajectory and prior exposure. It presents three studies that examine improved phenotypes based

on observable transition points in smoking cessation with appropriate prior exposure in relation to numerous variables for smoking behavior and comorbid conditions.

Conclusions

1. More tightly defined phenotypes of smoking behavior that are based on transitions along the smoking trajectory and adequate prior exposure have the potential to reduce the classification bias and lack of specificity inherent in broader existing phenotypes such as current smoking status. These improved phenotypes, in turn, may lead to closer correlations between smoking behavior and genetic variables in future studies.
2. Studies involving both longitudinal and cross-sectional population data show measurable differences among improved phenotypes, including sustained quitters, relapsers, and never quitters, in key markers such as smoking history, other indices of nicotine dependence, and comorbid conditions such as psychological symptoms and alcohol use.
3. Refined nicotine-dependence phenotypes based on longitudinal characterizations of smoking patterns show promise for further testing in genetic studies in support of potential phenotype-gene causal associations for nicotine dependence. Research indicates the potential need for further refinement of such phenotypes.

Chapter 11. Incorporating Social Context into Genetic Studies of Nicotine Dependence

Chapter 11 examines the available research and future trends related to social context factors that could inform subsequent genetic studies of smoking. The chapter considers macrocontextual factors such as culture and socioregional factors, microcontextual factors such as smoking in specific interpersonal relationships, and integrated proximal indicators of both macro- and microcontext such as ecological momentary assessment.

Conclusions

1. Social context infl uences on developmental pathways to nicotine dependence reflect gene-environment interplay that comprises the elements of a traditional epidemiological framework including a host (e.g., smokers and genetic endowment), environmental factors (social network), and an agent (e.g., tobacco).
2. Macrocontextual factors such as culture, socioregional variables, and socioeconomic status can modify or even nullify genetic influences on nicotine dependence. For example, a twin study revealed a prevalence rate for smoking of less than 1% in Chinese women, reflecting an inhibitory cultural influence. Family or neighborhood socioeconomic status and density of tobacco sales outlets are examples of specific contextual factors that appear to influence smoking risk among adolescents.
3. Microcontextual approaches have revealed factors such as exposure to parental, sibling, and peer smoking that may moderate genetic influence on behavioral smoking measures. The genetically informative Nonshared Environment in Adolescent Development Project, which comprised twins as well as other siblings, indicated that sibling interaction patterns may moderate the shared environmental effects that influence adolescent smoking.
4. Studies of smoking behavior using ecological momentary assessment, designed to measure both macro- and microcontextual factors, show that smoking behavior varies with both location and companions. Such

assessments serve as a possible future model for incorporating integrated social context issues such as actual clinical and public health efforts to reduce tobacco use within etiological architectures.

5. Future work incorporating social context within gene-environment studies of smoking behavior and nicotine dependence will benefit from a greater focus on environmental factors, including more-fine-grained and comprehensive assessments of potential environmental influences.

Chapter 12. Using Ontologies in Hierarchical Modeling of Genes and Exposure in Biological Pathways

Chapter 12 examines the potential for the use of hierarchical modeling techniques within the framework of an ontology that quantifies relationships across genotypes and phenotypes for nicotine dependence. The chapter provides an overview of statistical approaches for genetic association studies in tobacco use, presents the results of a study of nicotine metabolism that shows significant genetic associations with nicotine clearance levels, discusses design and analysis considerations in the use of hierarchical modeling in conjunction with stochastic variable selection, and explores the use of ontologies for codifying prior knowledge to support efficient computational analysis of hierarchical models.

Conclusions

1. The available knowledge of nicotine dependence arises largely from studies that model the independent association of candidate genes with outcome measures. Such studies often fail to reflect the complexity of interacting factors and discrete events that can influence smoking behavior and, therefore, may not provide a clear picture of biological mechanisms affecting nicotine dependence.
2. A promising approach to the study of nicotine dependence involves the use of prior biological knowledge about the relations between genotypic and phenotypic variables in a hierarchical modeling framework. This allows prior knowledge to aid in estimating specific genotypic effects and to guide a stochastic search over all possible statistical models.
3. The use of ontologies is a promising new direction for the elucidation of the genetic basis of nicotine dependence. An ontology is a construct or model that represents entities in both genotypic and phenotypic domains as well as their interrelations. The use of an ontology permits the modeling of hierarchical relationships by using directed acyclic graphs spanning genotypes and endophenotypes and phenotypes, while taking advantage of prior knowledge to quantify these relationships, making them amenable to computational analysis.
4. A study of nicotine metabolism that used data from the Northern California Twin Registry to examine the total clearance of nicotine and the *trans* 3′-hydroxycotinine to cotinine ratio, with the Nicotine Pharmacokinetics Ontology as a framework, showed a significant association between specific polymorphisms for *CYP2A6* and measured nicotine clearance levels as well as statistically significant results for single nucleotide polymorphism 4 within *UGT1A4*.
5. Hierarchical modeling combined with the use of an ontology defining relationships between constructs of interest represents a promising area for further research in studying a possible genetic basis for nicotine dependence as well as for understanding

the interaction between genetics and social and environmental influences on tobacco use and dependence.

Part 6—Future Directions

Chapter 13. Future Directions

Chapter 13 starts with a discussion of how examining the genetics of tobacco dependence may affect basic and clinical research. It then outlines future research needs for topics covered in parts 2–5. The chapter ends by presenting higher level recommendations for future research in nicotine dependence that cut across the content of this volume. These volume level future directions were identified by the editors while preparing this monograph and after taking into account continuing developments in the field.

Crosscutting Issues

- A comprehensive approach to examining and reporting genotype-phenotype associations should be adopted; single-gene, single-variant association studies should be discouraged unless accompanied by reports of replication and validation.
- Researchers working in the field of genetics and nicotine dependence should be mindful of the potential for misinterpretation of results by lay audiences. Efforts to communicate results to the media should include the limitations of the work along with the extent to which the results are reliable and generalizable. Doing so will minimize the chances of stigmatizing subgroups in the population.
- An ontology-based approach to nicotine dependence, with specification of expected relations within and between phenotypic domains, will provide an interpretive context and more focused hypotheses for future research; this will lead to an ongoing refinement of the ontology as new information becomes available.
- A greater use of strategies that combine differing levels of analysis is needed. The incorporation of measured genetics into genetic latent growth curve and/or latent class models in extended twin designs, for example, will provide information on the extent to which variation in one or more genes plays a role in the overall estimate of genetic variation in any particular phenotype. In addition, a nicotine reward phenotype may be characterized via behavioral measures of self-administration, self-report assays, and imaging measures of activity in brain regions associated with reward processing. This, in turn, could spur the hunt for more genetic variants and gene-gene or gene-environment interactions to account for more of the overall genetic variation estimated in the biometric models. Inclusion of quantified life events, cultural factors, and extant clinical and public health efforts in tobacco control and prevention in genetic studies is also warranted.
- Genome-wide association analysis of phenotypes considered to be risk factors for the adoption or maintenance of nicotine dependence would lead to further understanding of the pathways by which children progress to adult nicotine dependence.
- Given the enormous social, health, and economic impacts of nicotine dependence, the coordinated effort of multiple research teams to address the many opportunities for further research identified in this volume is warranted.
- There is a need to examine the association between gene variants and phenotypes of relevance in both the presence and absence of environmental risk factors. Emerging evidence from longitudinal studies of adolescents suggests that genetic associations with indices of

nicotine dependence may be stronger and more robust when acting in the absence of environmental pressure to not use tobacco. Another way in which gene-environment interactions may influence nicotine dependence is during and/or following attempts to quit the use of nicotine-containing products. For example, variation in genes responsible for drug metabolism could interact with the dosing or duration of pharmacotherapy for nicotine dependence to reduce drug efficacy. A third possibility for further exploration of gene-environment interactions involves the period following smoking cessation. The relationship between genetic variation and the likelihood of relapse back to nicotine dependence could well be dependent on the presence of conditioned cues to smoke or environmental stress.

- Epigenetic methodologies promise to further understanding of the impact of the environment on the differential expression of gene variants. One possible approach, described in chapter 2, involves the comparison, at the genomic and/or expression level, of lymphoblastoid cell lines from identical twins discordant for nicotine dependence or other characteristics such as nicotine metabolism. Informative measures of environmental exposures will enhance the power of this approach to account for monozygotic twin discordance.
- Much of the tobacco literature examines genetic susceptibility to smoking initiation and cessation only among very broad groups, without an understanding of the complexities or variations within these categories in patterns of smoking behavior. Combining very different subgroups of smokers into a few common phenotypes and then using such heterogeneous groups in research studies may be hindering progress in understanding the role of genetics in complex behaviors such as smoking. Moreover, standard definitions of smoking behavior from epidemiological surveys are not commonly used, making it difficult to compare results among genetics studies and to put these results into the context of knowledge gained from other disciplines. Therefore, researchers should be encouraged to use existing standardized definitions and measures of tobacco use behavior and to examine the role of genetics and environment in a greater number and broader range of more homogeneous groups of tobacco users.
- Epidemiologists and surveillance researchers should be encouraged to contribute more to the conceptualization, identification, definition, and operationalization of potential phenotypes of tobacco use behavior and then to demonstrate the utility, reliability, and validity of these potential phenotypes by using data from representative national surveys.

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