13

Future Directions

Although a predominant scientific theory exists on how nicotine leads to dependence, further study is needed to explore the relationships between genetic, environmental, and social influences on dependence. This concluding chapter summarizes how tobacco genetics may affect basic and clinical research and provides summaries and recommendations for each part of the monograph and cross-cutting recommendations for the entire volume.

- *Continued research on genetics may enhance the understanding of how nicotine's positive and negative effects lead to smoking relapse and nicotine dependence, the role genetic variation plays in acquiring dependence, and how to develop more effective treatments for nicotine dependence.*
- *The next generation of nicotine-dependence investigators are encouraged to conduct research at varying levels of analysis and approaches to studying genotype-phenotype associations, take into account the environmental context as well as G×E interactions, use a broader range of more homogenous groups of tobacco users, and to clearly communicate their results to lay audiences and the media in ways that will not be used to stigmatize subgroups of the population.*

This research has promise to refine existing nicotine-dependence treatments and identify new ones. Understanding the role of genetic susceptibility to nicotine dependence within the context of what is already working in the field of tobacco control should help to design and implement more effective treatments for nicotine dependence and enhance tobacco prevention and control policies.

Introduction

This chapter begins with comments on how continued research in the area of genetics and nicotine dependence may influence future research at the basic and clinical levels by enhancing our understanding of the involvement of dopaminergic pathways responsible for nicotine dependence, the role of genetic variation in the initial acquisition of nicotine dependence, and the development of more effective treatments for nicotine dependence. The second portion of the chapter provides summaries and recommendations from parts 2–5 of the monograph and concludes with several crosscutting suggestions for the future.

Genetics and Nicotine Dependence: Implications for Basic and Clinical Research

Nicotinic and Dopaminergic Receptors

Tobacco smoke contains more than 5,000 compounds (many of which are of unknown impact with regard to dependence), and nicotine is widely considered to be the most addictive of these.[1] The predominant theory concerning how nicotine leads to dependence posits that acute nicotine binds to nicotinic receptors located on dopaminergic neurons in the ventral tegmental area (VTA) of the substantia nigra. The resultant dopamine release is associated with the experience of pleasure and the enhancement of some cognitive functions, such as sustained attention and vigilance, through neuronal projections from the VTA to the nucleus accumbens, frontal cortex, and striatum. Unfortunately, for the chronic tobacco user, long-term use results in a reduced function of nicotinic and dopaminergic receptors and more nicotine is required to maintain the same effects on mood and cognition. At the same time, cues in the environment become conditional triggers to smoke. When a regular tobacco user attempts to quit, withdrawal from nicotine is associated with a concurrent upregulation of nicotinic receptors and downregulation of dopaminergic receptors thereby leading to unpleasant symptoms, dysphoria, and in some cases, a wide array of cognitive decrements. The simultaneous avoidance of negative symptoms (negative reinforcement) and the pursuit of the positive effects of nicotine (positive reinforcement) lead to a harmful and recurring cycle of relapse back to smoking.[2] Because nicotinic and dopaminergic receptors are but two of many pathways (such as glutamatergic, opioid, and serotonergic) implicated in the neurobiology of dependence, the complete picture of reward and dependence is undoubtedly much more complex and likely involves second-messenger systems.

A number of lines of evidence support the dopamine hypothesis of dependence. Various abused drugs, including nicotine, result in measurable and, in some cases, substantial increase in dopamine in terminal dopaminergic fields, particularly the nucleus accumbens.[3] Significant changes in regional cerebral blood flow have been observed in the nucleus accumbens, hippocampus, and orbitofrontal cortex of smokers in response to the first cigarette of the day following overnight abstinence.[4] In individuals with nicotine dependence, cue-induced changes dependent on blood oxygen level in brain regions along major dopaminergic pathways provide further neuroanatomical support to the hypothesis.[5] Continued advances can be expected in the elucidation and validation of the dopaminergic basis for nicotine dependence as new phenotypic measures, such as those observed with functional

magnetic resonance imaging, are examined in relation to novel genetic variants.

Nicotine Dependence—A Note on Developmental Pathways

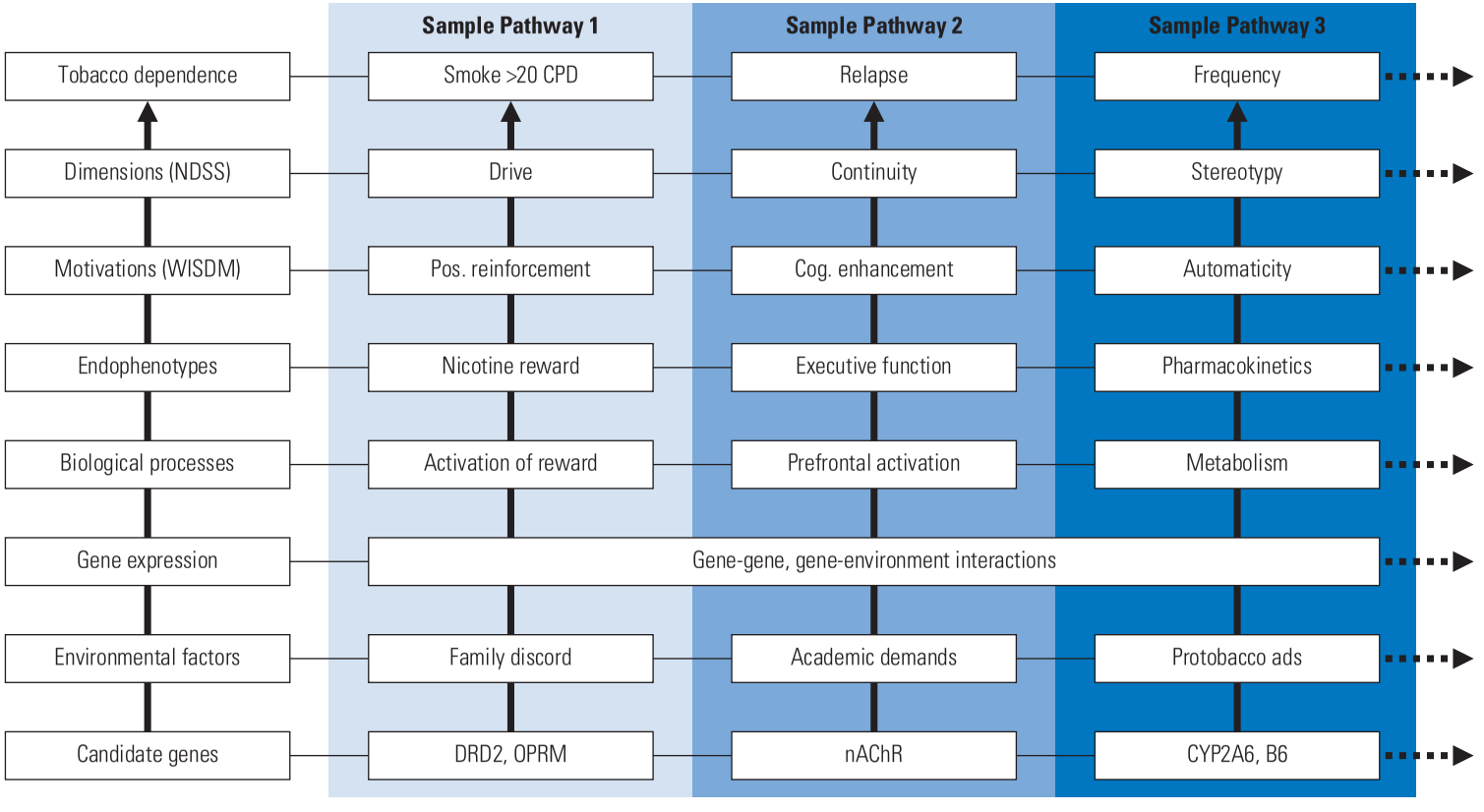
In addition to strides made in understanding the basic neurobiological pathways to nicotine dependence, evidence indicates that the neurobiological and social processes by which young people exposed to tobacco products become addicted to nicotine are likely to be different from those responsible for the maintenance of dependence. Because the developing child's brain is dynamic, it is possible that the brains of young people may be more susceptible than those of adults to the addictive properties of nicotine. For example, as the brain matures, the amount of gray matter on the cortical surface of the brain decreases from back to front as synaptic pruning occurs.[6] It has also been shown that frontal and temporal lobe volumes are smaller in adolescents than in adults.[7] Evidence from animal models shows that preadolescents, as compared with older adolescents, show increased upregulation of nicotinic receptors (including α5 and α6, and β2) and increased nicotine self-administration following preexperimental exposure to nicotine.[8] This suggests that age-gene-environment interactions may be operating to heighten the risk for entering into a tobacco use trajectory that ultimately leads to chronic tobacco use in adulthood. The use of developmental trajectories to describe how different people become dependent as adults (see chapters 4–6 in this volume) is a new area of research receiving increased attention.[9,10] In the future, it will be possible to examine genomic differences among trajectory subgroups.

Genomic Studies of Nicotine Dependence in Adults

Early work involving twins suggested that genetic factors may account for more than 50% of interindividual variation in smoking initiation and nicotine dependence. Subsequent studies have sought to understand how genetic variation may affect the underlying neurobiology of nicotine dependence.[9] New analytic capacity will permit more powerful efforts to identify genetic variants key to nicotine dependence and its treatment. More than 25 whole-genome linkage scans involving nicotine dependence (and/or related phenotypes, such as number of cigarettes smoked per day and maximum cigarettes ever smoked in a single day) have been reported. A study in 2006[11] identified a linkage peak on chromosome 6 for scores from the Fagerström Test for Nicotine Dependence (FTND).[12] Swan and colleagues[11] also found suggestive linkage peaks on chromosomes 8 (nicotine dependence related to criteria of the *Diagnostic and Statistical Manual of Mental Disorders*)[13] and 15 (report of a previous reason for relapse of "enjoyed smoking too much"). All three peaks are near candidate genes of interest in the nicotinic and opioid pathways. Significantly, a genome-wide association study in 2007 of 32,000 single nucleotide polymorphisms (SNPs) in nicotine-dependent cases (n = 1,050; FTND score more than 4 when smoking at maximum) and nondependent controls (n = 879; smoked at least 100 cigarettes lifetime but had a score of 0 on the FTND) provided evidence that some of the strongest associations were with variants in nicotinic, opioid, and dopamine genes, several of which were close to candidate chromosomal regions.[14,15] What is apparent from this work is that convergence across studies and methodologies is now being seen.[16] Great progress will continue to be made in identifying specific gene variants that play a role in nicotine dependence.

Figure 13.1 represents a schematic of what the future might hold as the pieces of the puzzle of nicotine dependence are identified, certified, and assembled. On the

Figure 13.1 Some Examples of Hypothetical Gene to Phenotype Pathways



Note. CPD = cigarettes smoked per day; NDSS = Nicotine Dependence Syndrome Scale (Shiffman, S. A. Waters, and M. Hickcox. 2004. The Nicotine Dependence Syndrome Scale: A multidimensional measure of nicotine dependence. *Nicotine & Tobacco Research* 6 (2): 327–48.); WISDM = Wisconsin Index of Smoking Dependence Motives (WISDM-68) (Piper, M. E., T. M. Piasecki, E. B. Federman, D. M. Bolt, S. S. Smith, M. C. Fiore, and T. B. Baker. 2004. A multiple motives approach to tobacco dependence: The Wisconsin Inventory of Smoking Dependence Motives (WISDM-68). *Journal of Consulting and Clinical Psychology* 72 (2): 139–54.). From Swan, Lessov-Schlaggar, and Brigham, 2005, SRNT Annual Meeting.

far left of the figure is the arrangement, in ascending order, of components of a pathway that begins with the impact of candidate genes through their expression in response to the environment (e.g., tobacco, tobacco advertising, peer or parental smoking, cigarette design, or other environmental pressures to use or not use tobacco). The result of the gene-environment interaction is to initiate a biological process that can be measured in some fashion (e.g., nicotine metabolism, reward, cognition)—that is, the endophenotype. The biological process, in turn, must contribute in some way to motivate an individual to continue smoking. While the primary motivations to smoke may be different across individuals, they must, in turn, be associated with any or all dimensions of nicotine dependence and, finally, to observable tobacco use behavior.

One of the key features in this progression of events is that every step in the path is potentially measurable. Another feature is the implied association between components of the pathway, testable through a planned series of experimental studies to determine the validity of the pathway. The temporal sequencing of components in the pathway to confirm causality could be investigated through the use of any of a variety of tools. Finally, the arrows extending through the rows of the diagram suggest a progression over time in which any individual, given his or her initial variation across a number of genes and subsequent variation across environments, could come to exhibit behavioral variation in a number of indices of nicotine dependence, including tobacco use trajectories.

Figure 13.1 provides three hypothetical pathways, each of which could be examined empirically. To illustrate, the first example begins with variation in genes involved in the reward pathway that are activated through interaction with an environment that provides few reinforcers. Individuals with this confluence of initial conditions would be expected to experience heightened reward from nicotine and acquire the need for sustained positive reinforcement—a motivation to continue to smoke. The need for sustained reinforcement would result in an increased drive to smoke and an increase in number of cigarettes smoked per day. The remaining two pathways, initiated through interaction between variation in the nicotinic receptors or metabolic genes, can be viewed as leading, ultimately, to different components of nicotine dependence. Investigators of the future should use this schematic, or one like it, to place their efforts in a theoretical context that permits empirical tests of hypothesized connections in a nicotine dependence pathway (see chapter 3 for another formulation of potential pathways and chapter 12 for an analytic approach for studying pathways). Such an approach will help others make sense of the plethora of results likely to continue to emerge over the coming years.

Future Directions

This part summarizes the discussions of the research presented in the monograph (parts 2 through 5) and suggests future research agenda items.

Part 2—Theoretical Considerations

Research presented in part 2 from investigators who examined both competing and distinct models of dependence demonstrated that nicotine dependence is multidimensional and that numerous theories on its development are available.

Guided by their own hypotheses about the nature, manifestation, and development of dependence, investigators recommended

that incorporating a more comprehensive portrayal of dependence development may help find the link between genes and behavior for genetic dependence susceptibility.

Part 2 of this monograph also examined issues surrounding the complex genetic and behavioral measures that combine and contribute to nicotine dependence gleaned through studies of inbred mouse strains. Available evidence used to translate the validity of the mouse findings to humans holds promise in coming to understand individualized responses to nicotine.

Additional areas recommended for future research include the following:

- Phenotypic assessments are needed that reflect the different stages in progression to dependence (intermediate and transitional phenotypes) (chapter 3).
- Future research should address the extent to which the different types of tolerance (e.g., acute or chronic, behavioral or dispositional) are related to core features of dependence, such as a pervasive pattern of drug use. Further understanding of the neural and genetic substrata of tolerance, and how these compare with other causal influences on dependence, may elucidate the role of tolerance in dependence development (chapter 4).
- Given the tremendous potential created by the availability of well-characterized mouse strains and both knockout and knockin preparations, the use of such tools is needed to explore genetic influences on phenotypes that provide additional insight into the processes involved in nicotine dependence. Additional assays, both physiological and behavioral, should be used to expand understanding of the genetic contributors to the critical motivational processes of dependence (chapter 4).

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

Over time, the paths that smoking behavior take vary widely. These patterns of development are an important basis for genetic studies of nicotine dependence. This third part of the monograph focused on issues related to studying these trajectories.

One chapter focused on the development of smoking patterns from adolescence (when smoking and other substance use are most commonly initiated) to adulthood. Data collected from related individuals were used to examine the association between smoking initiation and progression in an effort to understand the etiology of nicotine dependence. Genetic data indicating the existence of an overlapping developmental pathway between smoking and other substance use in identical and fraternal twins were also examined.

Suggested topics for continued study include the following:

- Future research should link hypothesized preexposure endophenotypes to trajectories that might constitute dynamic phenotypes of cigarette smoking. These studies should also consider other forms of tobacco use (chapter 5).
- Better specification is needed of the relation between trajectories of smoking behavior and the development of nicotine dependence, as well as the relation between adolescent and adult trajectories. Moreover, further research (using animal and human models) is required to understand the mechanisms underlying age-specific effects of initial nicotine exposure, which have shown a significant relation between an early age of onset and steeper acceleration over time (chapter 5).

- It is important to determine whether a particular individual feature of a trajectory (e.g., age of onset or steepness of acceleration) is the important phenotype or whether it is more useful to consider an entire trajectory group. Moreover, different research approaches are needed to determine whether phenotypes are best considered as categorical "groups" or as representations of an underlying continuous dimension. Future research will help determine if there are important ethnic differences in these groups (chapter 5).
- Efforts should be made to develop reliable and valid methods to retrospectively reconstruct trajectories in addition to pursuing a range of longitudinal study designs (chapter 5).
- 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 (chapter 6).
- Future genetic research should jointly examine the extent to which different trajectories (combinations, of course) and the use of multiple substances (comorbidity) are genetically influenced. If it can be shown that phenotypes represented by broader substance-use trajectories are equally or more heritable than are single-substance trajectories, both phenotypic and genetic work can proceed more efficiently. Findings would have implications for whether researchers should take a more genetic approach in preventing and treating substance-use disorders (chapter 7).

Part 4—Endophenotypes

Characteristics present at or before exposure to nicotine may help to identify individuals genetically susceptible to nicotine dependence. Similarly, measures in smoking persistence may help predict the success of cessation attempts among chronic smokers.

Part 4 of the monograph focused on data indicating that genetic risk for nicotine dependence may also be affected by the presence of several psychological factors, such as approach-, avoidance-, and control-related smoking risk variables, at or prior to smoking and nicotine exposure. Chronic smokers were also a focus. Data presented indicate that the path to persistent smoking should include not only measurement of genetic factors but also motivational, sensory, cognitive function, craving, and other behavioral elements.

Additional areas recommended for future research include the following:

- Higher-order trait domains (approach, avoidance, control, and affiliation/empathy) all have some promise in relation to smoking risk. However, these traits are best understood in relation to lower-level neural systems, which, in turn, point to more molecular cognitive or physiological measures that can be examined as endophenotypes. More research is needed to evaluate a range of context-sensitive physiological measures as candidates of these lower systems (chapter 8).
- There is great potential for future research to provide the evidence for or against the criteria important for endophenotype measures of nicotine dependence and to inform the debate about the utility of endophenotypes in genetic research (chapter 9). Differences in quitting motivation between laboratory research participants and smokers in clinical studies may impede the development and validation of brief laboratory-based behavioral procedures that may serve as endophenotypes. Future endophenotypic research should

either be designed to examine the impact of these motivational differences or should exclude non-treatment-seeking smokers (those not trying to quit or with no interest in quitting) (chapter 9).

Part 5—Epidemiological and Methodological Considerations

In part 5 of the monograph, methodological and epidemiological issues related to the future direction of genetic studies on nicotine dependence are discussed. An epidemiological approach for modeling smoking phenotypes, models that incorporate social context factors, and hierarchical modeling techniques were presented.

Using an epidemiologic approach to defining smoking phenotypes, three analyses were presented that demonstrated that using more tightly defined comparison groups would yield more consistent findings about the role of genetics in smoking behavior. Other analyses that incorporated social context into genetic studies of nicotine dependence indicated that genetic susceptibility to smoking may be influenced by the social context and environment—such as having peers, parents, and siblings who smoke. How the genetic variation affects the analysis and interpretation of the role of genetics in tobacco use closes out this part of the monograph.

Results from a pilot study using hierarchical modeling techniques demonstrated that formally incorporating different phenotypes and genotypes into the statistical analysis may help to lessen some of the difficulties experienced in evaluating the numerous factors affecting nicotine metabolism.

Research agenda items for future consideration include:

- Researchers should be encouraged to use more tightly defined phenotypes of smoking behavior that are based on transitions on the smoking trajectory and adequate prior exposure, as these have the potential to reduce misclassification bias and the lack of specificity inherent in broader existing phenotypes such as current smoking status (chapter 10).
- There may be etiological heterogeneity in the mix of genes and environments that can be captured only by incorporating candidate social contextual measures in genetically informative designs. To understand the mechanisms underlying such etiological heterogeneity, researchers should be encouraged to examine a broad number of both macro- and microsocial factors (i.e., conduct a "whole environment" scan) (chapter 11).
- Evaluating sources of etiological heterogeneity may help in understanding the mechanisms by which endophenotypes become salient for smoking behaviors under specific environmental conditions but not others. Therefore, future research should use advanced measurement of both endophenotypes and social contexts to potentially illuminate core environmental factors that dwarf individual-level propensities as well as highlight especially prominent endophenotypes that convey risk under particular environmental conditions (chapter 11).
- It is becoming increasingly untenable to ignore social contextual factors without sacrificing a broader and more comprehensive understanding of the etiological architecture of complex phenotypes such as nicotine dependence. Therefore, if the field is to take seriously the proposition that gene-environment interplay will play a key role in eventually understanding the mechanisms by which genes contribute to smoking behavior and nicotine dependence, a dedicated effort will be needed not only to incorporate environmental measures with more regularity and vigor but

also to invest the time, resources, and collaborative expertise necessary to provide the best available data on the environment (chapter 11).

- Since complex traits are the results of many factors acting in concert, statistical analysis needs to be rich enough to identify sets of factors acting synergistically. One approach is to use ontologies in hierarchical modeling in conjunction with stochastic variable selection for future genetic analyses of tobacco use.

Crosscutting Issues for Future Research in Nicotine Dependence

In developing this monograph and examining continuing developments in the field, the editors identified several higher level recommendations for future research in nicotine dependence that cut across the content of this volume.

- 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.

References

1. Fowles, J., and E. Dybing. 2003. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tobacco Control* 12 (4): 424–30.
2. Koob, G. F., and M. Le Moal. 1997. Drug abuse: Hedonic homeostatic dysregulation. *Science* 278 (5335): 52–58.
3. Di Chiara, G., and A. Imperato. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America* 85 (14): 5274–78.
4. Zubieta, J. K., M. M. Heitzeg, Y. Xu, R. A. Koeppe, L. Ni, S. Guthrie, and E. F. Domino. 2005. Regional cerebral blood flow responses to smoking in tobacco smokers after overnight abstinence. *American Journal of Psychiatry* 162 (3): 567–77.
5. Smolka, M. N., M. Buhler, S. Klein, U. Zimmermann, K. Mann, A. Heinz, and D. F. Braus. 2006. Severity of nicotine dependence modulates cue-induced brain activity in regions involved in motor preparation and imagery. *Psychopharmacology (Berl)* 184 (3–4): 577–88.
6. Gogtay, N., J. N. Giedd, L. Lusk, K. M. Hayashi, D. Greenstein, A. C. Vaituzis, T. F. Nugent 3rd, et al. 2004. Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America* 101 (21): 8174–79.
7. Sowell, E. R., P. M. Thompson, C. J. Holmes, T. L. Jernigan, and A. W. Toga. 1999. In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. *Nature Neuroscience* 2 (10): 859–61.
8. Adriani, W., S. Spijker, V. Deroche-Gamonet, G. Laviola, M. Le Moal, A. B. Smit, and P. V. Piazza. 2003. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. *Journal of Neuroscience* 23 (11): 4712–16.
9. Lessov-Schlaggar, C. N., M. L. Pergadia, T. V. Khroyan, and G. E. Swan. 2008. Genetics of nicotine dependence and pharmacotherapy. *Biochemical Pharmacology* 75 (1): 178–95.
10. Karp, I., J. O'Loughlin, G. Paradis, J. Hanley, and J. DiFranza. 2005. Smoking trajectories of adolescent novice smokers in a longitudinal study of tobacco use. *Annals of Epidemiology* 15 (6): 445–52.
11. 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.
12. Heatherton, T. F., L. T. Kozlowski, R. C. Frecker, and K. O. Fagerström. 1991. The Fagerström Test for Nicotine Dependence: A revision of the Fagerström Tolerance Questionnaire. *British Journal of Addiction* 86 (9): 1119–27.
13. American Psychiatric Association. 2000. *Diagnostic and statistical manual of mental disorders: DSM-IV-R.* 4th ed. text rev. Arlington, VA: American Psychiatric Publishing.
14. 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.
15. 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.
16. Uhl, G. R., T. Drgon, C. Johnson, O. O. Fatusin, Q. R. Liu, C. Contoreggi, C. Y. Li, K. Buck, and J. Crabbe. 2008. "Higher order" addiction molecular genetics: Convergent data from genome-wide association in humans and mice. *Biochemical Pharmacology* 75 (1): 98–111.