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

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The search for a possible genetic basis for nicotine dependence requires constructs that serve as a link between genes and behavior. Common existing measures of nicotine dependence are highly heritable and have high predictive validity for smoking outcomes yet lack specificity relative to the underlying biological mechanisms that could inform future genetic research. This chapter examines theoretical issues in establishing nicotine-dependence phenotypes, including

- *Distal measures of nicotine dependence, such as the Fagerström tests, the* Diagnostic and Statistical Manual of Mental Disorders, *and others, focusing on mature nicotine dependence*
- *Newer multidimensional measures of nicotine dependence, such as the Nicotine Dependence Syndrome Scale and the Wisconsin Inventory of Smoking Dependence Motives, examining motivational factors leading to dependence*
- *Endophenotypes and transitional phenotypes, measuring quantities before and after nicotine exposure, respectively, that may potentially form a causal path between specific genetic actions and measures of nicotine dependence, including cognitive, affective, and craving factors*

Further study is needed to establish the validity of such endophenotypes and transitional phenotypes for upstream measures of nicotine dependence and their relationship with genetic and gene-environment influences, which, in turn, may support further research on the impact of such influences on smoking outcomes and behavior.

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Introduction

This chapter examines a theoretical basis for the assessment of phenotypes for nicotine dependence, focusing on strategies that investigators might use to assess nicotine dependence with the goal of uncovering its genetic bases. First, an epistemological system (i.e., construct validation) is discussed for studying such complex constructs as dependence. This system provides a vocabulary, a set of principles, and an inferential basis for evaluating evidence for the assessment of nicotine dependence. In addition, a conceptual model is presented that shows how dependence assessments may be characterized by their specificity and their proximity to genetic variants and the biological mechanisms that the variants directly express. This model reveals that genetic variants may be related to a developmental progression of phenotypes: those preceding nicotine exposure (intermediate or endophenotypes), those that arise out of initial nicotine exposure but precede frank dependence (transitional phenotypes), and those regarded as mature clinical phenotypes.

Next, the chapter reviews evidence on existing measures of dependence, including both traditional diagnostic measures and newer multidimensional measures. This evidence is used to draw inferences about the nature and structure of dependence, especially as it manifests in long-term, heavy smokers. Then, existing data are used to address general questions about strategies for genetic mapping. These questions include whether different types of assessments need to be used for different smoker subpopulations, which particular measures need to be used to assess the core and breadth of the phenotype, and how to model environmental influences in such mapping. Then, the chapter addresses future directions for phenotypic assessment, especially the need to develop assessments that reflect the different stages in progression to dependence (intermediate and transitional phenotypes). Finally, issues regarding the integration of phenotypic measures with research design and analytic strategies are addressed.

Appropriate and accurate assessment of the nicotine-dependence phenotype construct is needed to understand better the molecular genetic basis of tobacco use and nicotine-dependence. The nicotine-dependence *phenotype* comprises the measurable manifestations of heritable information that result in nicotine use that produces persistent socially, clinically, or medically significant distress or dysfunction. Although the use of any sort of nicotine delivery system might satisfy this criterion, this review will concentrate on research and theory relevant to the smoking of tobacco cigarettes.

Most measures of nicotine dependence (e.g., diagnostic items) assess general features of dependence that are causally distal to underlying genetic influences. Common distal measures such as the Fagerström tests and the criteria of the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition *(DSM-IV)*[1] tend to assess mature states of nicotine dependence. Such measures could mask upstream causal factors that may, in fact, be closer to a genetic basis for nicotine dependence. Nevertheless, these distal measures assess constructs or dimensions that are highly heritable. Examining newer multidimensional criteria for nicotine dependence, as well as other approaches that take more of a causal and developmental perspective, can serve as an important key to developing phenotypes, endophenotypes, and intermediate phenotypes that, in turn, may correlate more closely with gene action.

The need to assess the phenotype accurately is patent. Genetic variants, by themselves, are relatively uninformative. They attain greater information value to the extent

that they are associated with biological, behavioral, cognitive, or clinical outcomes of interest. Thus, the phenotype confers clinical, societal, and/or theoretical meaning on such genetic variants as alleles. For this reason, phenotypic assessment is central to uncovering the genetic substrata relevant to the maintenance of chronic tobacco use and dependence. However, the attempt to measure this phenotype well is a daunting task that demands that investigators have a clear idea of what they want to measure and why.

One basic question that investigators must address is whether they are interested in "nicotine dependence" as opposed to "tobacco dependence." This decision could have implications for the selection of genetic variants and phenotypic measures. For example, if one is interested in tobacco dependence, one might assess orosensory perceptual processes that could influence a person's gustatory reaction to tobacco. In addition, an investigator must decide whether to focus on researching "chronic tobacco use" or "dependence." The two terms refer to constructs that are related to one another, but are nevertheless distinct, and have important implications for dependence assessment. The focus in this chapter is largely on the assessment of nicotine dependence per se.

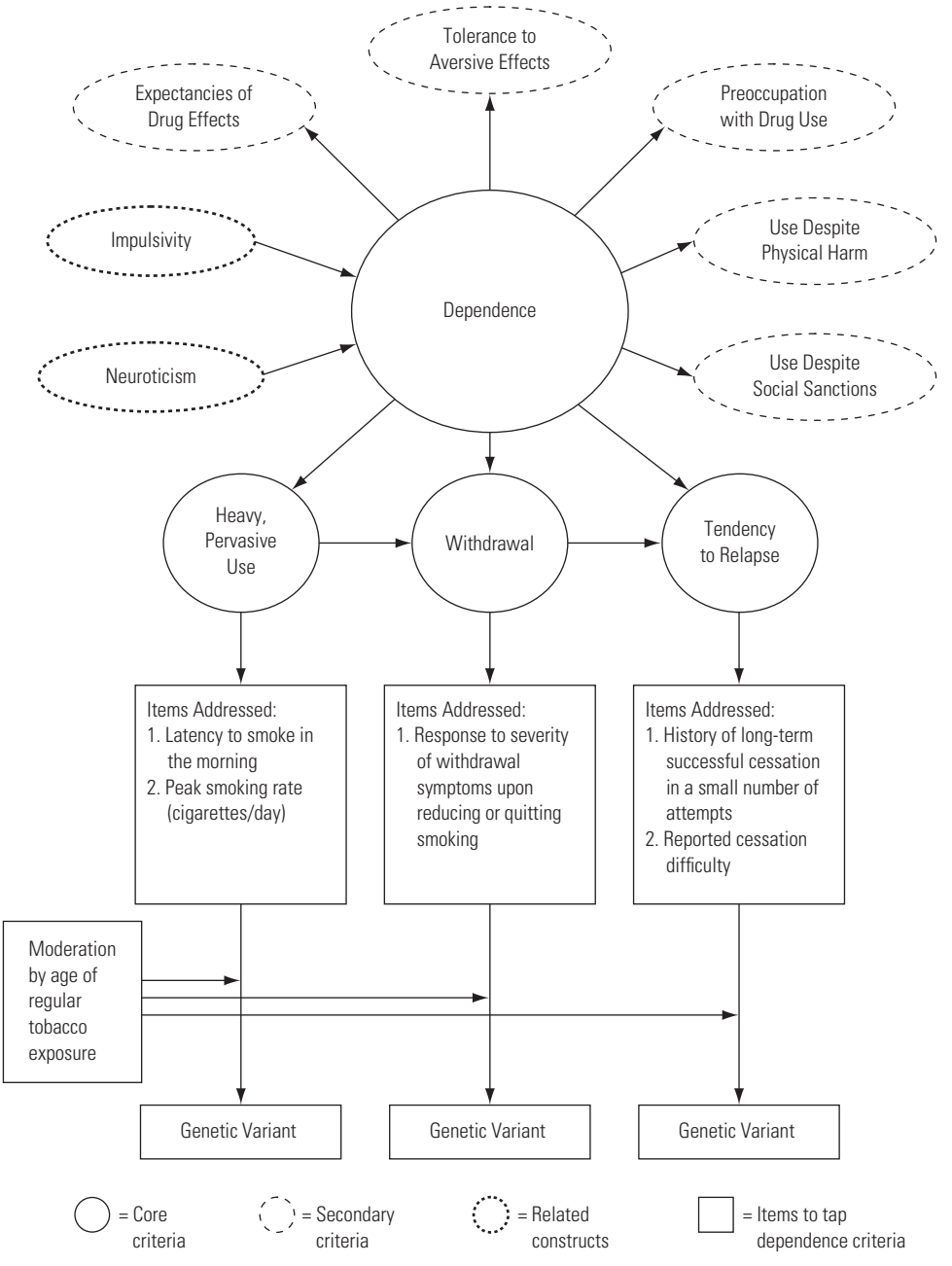
Construct Validation

The term *dependence* denotes hypothetical variables or constructs. A *construct* has been defined as "some postulated attribute of people, assumed to be reflected in test performance."[2(p283)] *Test performance* is broadly defined and may comprise any characteristic or behavior of the person that can be measured and that is thought to reflect the construct. Thus, the hypothesized features of nicotine dependence should be designed to explain a set of observations that are thought to constitute important outcomes or manifestations of dependence. These outcomes might include the difficulty that smokers have in quitting, the escalation of smoking over time, craving and withdrawal, and so on. The process of attempting to both uncover the nature of a construct and accurately measure it is known as *construct validation.* This chapter uses the construct validation approach as an interpretive structure or metatheory to guide a discussion of the available research and theory regarding nicotine dependence.

The construct validation approach typically starts with a set of behaviors or outcomes that an investigator wishes to explain. The behaviors or outcomes are usually of social or clinical importance and serve as *criteria* in construct validation research (figure 3.1). The investigator then hypothesizes a set of features and processes that seem to account for the outcomes; these are *construct properties.* Finally, the investigator must select or develop two sorts of assessments: those that measure the construct properties sensitively (i.e., the assessment instrument) and those that tap the outcomes of the construct. Thus, the construct validation approach involves identifying (1) a set of behaviors or outcomes to be explained (the criteria), (2) hypothesized features or processes that are thought to produce the outcomes (these features or processes are actually the mechanisms of the targeted construct, i.e., dependence), and (3) measurement strategies that accurately assess the construct (processes) and its manifestations (i.e., the criteria; figure 3.1). The construct validation approach should have two important payoffs: it should inform people as to the nature of the construct, and it should simultaneously allow them to measure the construct accurately.

A construct validation approach is most needed when there is no single adequate measure of an entity;[3] in such cases, the investigator must use multiple measures as

Figure 3.1 Nomological Net: Evaluation Context for a Model of Dependence and Its Relation to Genetic Variants



a means of estimating a person's standing on the targeted construct. The notion is that agreement or consensus across a group of related but imperfect indicators will yield better construct estimates than would the use of any single measure. Thus, a construct is a *latent variable* inferred from variables that can be directly observed (manifest variables—that is, the construct assessment or test). There would be no need to assess nicotine dependence as a latent variable if one could directly measure the pathological processes that cause it. For example, a definitive diagnosis of hypertension can be made in response to elevated blood pressure. In the absence of direct assessment of disease processes or pathognomonic signs, multiple converging measures can enhance diagnostic inferences.

The construct validation approach requires several distinct but interrelated questions to be addressed. For example, what are the criteria or outcomes that a nicotine-dependence measure should be able to predict? Just as the construct of gravity is invoked to explain the behavior of falling bodies, how can nicotine dependence be designed to explain certain clinical and societal phenomena? Figure 3.1 provides examples of core and secondary criteria that a model of nicotine dependence might comprise. Core criteria are those that are societally and clinically essential for the construct measures to explain (account for); secondary criteria are those that may provide useful information about the construct but are of somewhat lesser importance. The model depicted in figure 3.1, as an example, posits that, although nicotine dependence should be reflected in positive expectations about nicotine effects, the model's ability to predict relapse is more important.

A construct validation approach is a theory-based approach to epistemology. An investigator should select a dependence measure (items on a test) that accords with the investigator's theory of dependence: it would measure those variables that reflect, in the investigator's opinion, the presence and magnitude of the critical underlying features or mechanisms of nicotine dependence. The theory also should explain why those hypothesized features or processes affect both the dependence measure as well as the criteria. In sum, in the construct validation approach, a test is a measure of a mechanism or cause, such as a measure of blood pressure, that predicts and explains a person's status on a set of socially or clinically important criteria (e.g., risk of stroke, heart disease, need for treatment). And, if the measure of the mechanism does indeed predict the criteria, the researcher not only validates the test but also simultaneously supports the theoretical model of the studied disorder. Finally, the researcher would like to see that the test has discriminative validity; that is, it is most sensitive to the particular construct that is targeted (nicotine dependence) and less sensitive to related, but not central, constructs (e.g., regular smoking, problems caused by tobacco use).

Note that the construct validation approach is somewhat different from alternative approaches that view addiction as a social construction that cannot be verified or evaluated on the basis of relations with objective criteria.[3] In the customary treatment, addiction and dependence are viewed as equivalent to one another (but not equivalent to *physical dependence,* which is inferred from a withdrawal syndrome). Moreover, this treatment recognizes that dependence is a construction based upon social and theoretical perspectives, as does the approach advocated by West.[3] The construct validation approach, however, illustrates how to evaluate the validity of one's strategy for measuring dependence on the basis of the empirical relations stipulated by a guiding theoretical model. Therefore, it permits different investigators to hold very different views of dependence,

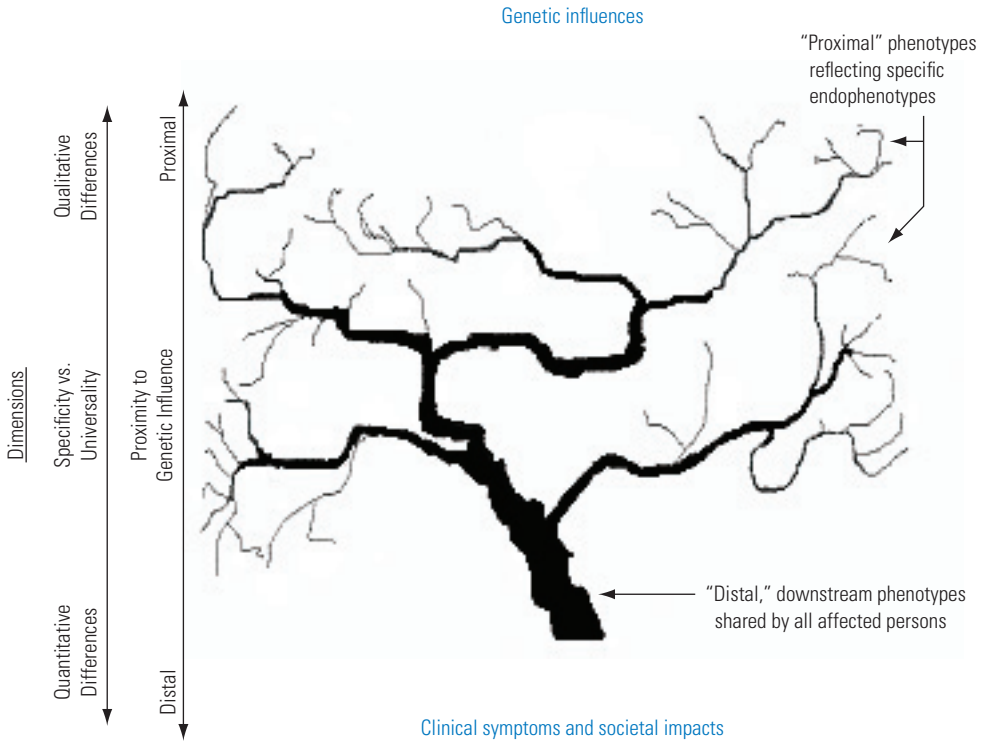
but at the same time, it provides a logical system for the simultaneous evaluation of both the theoretical and measurement models of dependence.

Most attempts to measure nicotine dependence have not used a formal construct validation approach. The field has, in general, attempted to measure nicotine-dependence criteria directly rather than attempting to measure the mechanisms or processes of nicotine dependence. For example, this is the approach modeled by the *DSM* and other diagnostic assessments. Such syndromal assessments are atheoretical and tend to focus on observable outcomes of a disorder. One reason for this approach is, no doubt, that investigators had only inchoate notions of dependence mechanisms or features and thus were unprepared to assess dependence mechanisms more directly. In addition, the purpose of such clinical instruments typically is to detect suffering and compromised function, not to assess causal influences. Regardless of the reasons, investigators have usually tried to measure general outcomes or criteria of severe nicotine dependence rather than mechanisms.

Distal Measures of Dependence

Figure 3.2 depicts a "watershed" model of disorder. This model assumes that different etiologic paths may lead to clinical levels of symptomatology. Approaches may focus on end points that vary in terms of their proximity to specific genetic variants in the causal chain of the disorder. This model will be discussed in greater detail later.

Figure 3.2 Watershed Model of Gene-to-Phenotype Influence



At this point it is worth noting that most nicotine-dependence assessments, thus far, have focused on assessing the "mature" or *distal* nicotine-dependence phenotype; that is, they have measured phenomena that are, no doubt, distant from, and nonspecific to, many of the underlying causal processes (including genetic variants) that contribute to the disorder. This has important implications for how nicotine dependence and genetic influences on it are viewed.

As noted, most research on nicotine dependence has used self-report measures that tap distal end products of dependence processes (e.g., smoking a great deal, being unable to quit) that have social and clinical import. These measures include scales contained in nosologies such as the *DSM,* as well as brief questionnaires such as the Fagerström Test for Nicotine Dependence (FTND).[4] In addition, researchers have used subsets of items or individual items from such scales (e.g., the Heaviness of Smoking Index [HSI]).[5] Such measures may have poor signal-to-noise ratios relative to biological mechanisms that underlie nicotine dependence and that may sensitively reflect status on particular genetic variants; that is, they may reflect numerous influences and are most likely causally remote from basic biological mechanisms of nicotine dependence (see figure 3.2). Despite these limitations, such distal measures have yielded clues as to the nature of nicotine dependence and its genetic influences.

The distal measures considered initially in this section (e.g., the FTND and psychiatric diagnostic criteria such as those in the *DSM-IV*[1*] elicit information regarding the general consequences or characteristics of nicotine dependence. Thus, these measures elicit information about how much people smoke, whether they experience withdrawal symptoms or craving, whether they tend to return to tobacco use once they stop, whether they have trouble controlling tobacco use, and so on. These measures were designed to capture major clinical manifestations of addiction,[3] not to assess features of dependence with strong genetic association.

Fagerström Measures

The Fagerström Tolerance Questionnaire (FTQ),[6] and measures derived from it, were intended to be unifactorial measures of nicotine dependence. These measures make up the FTQ itself, as well as the six-item FTND,[4] and the two-item HSI.[7] These measures are based on the construct of physical dependence, which was hypothesized to include facets such as the need to smoke early in the morning to alleviate overnight withdrawal, the need to smoke numerous cigarettes per day, and the invariance of smoking behavior—that is, smoking even when one is ill.[6]

Two questions on the FTND (i.e., questions 1 and 4) and the two questions of the HSI assume a pattern of daily smoking. It is very likely that scores on these items will have reduced validity if used with nondaily smokers.

Compared with the FTQ, the FTND has demonstrated better psychometric properties such as internal consistency.[8–10] However, these improved reliability coefficients are still low[8,11] and are below traditionally accepted standards for clinical use ($\alpha = .80$).[12]

Some studies show that the FTND has a two-factor structure, suggesting that it does

[*]The Cigarette Dependence Scale is also designed to assess a single factor of dependence. (See Etter, J. F., J. Le Houezec, and T. V. Perneger. 2003. A self-administered questionnaire to measure dependence on cigarettes: The cigarette dependence scale. *Neuropsychopharmacology* 28 (2): 359–70.) At present, there is too little evidence on this scale to permit its evaluation with regard to genetic mapping.

not measure a unitary construct of physical dependence.[8,9,13–16] Factor analytic research tends to show that even if more than one factor is obtained, the two factors are highly correlated.[13,17] Interitem correlations also reveal that not all items are highly related (r = .06–.39).[18] The various factor analytic studies differ in terms of factor-item linkages.[8,13,16] However, the weight of the evidence suggests the existence of two factors, with one of the factors suggesting a pattern of compulsive smoking, and the other factor reflecting what is termed "morning smoking" (e.g., whether one smokes more in the morning than at other times). The items that typically load on the compulsive smoking factor are those that assess the number of cigarettes smoked per day, time to first cigarette, and difficulty refraining from smoking when ill. There is some variability in which specific items load on this compulsive smoking factor, no doubt because the factors are intercorrelated and some items are highly correlated with both factors.[13,16] What is clear is that, in general, the first principal component or main factor is the compulsive smoking factor and that it accounts for the lion's share of predictive validity of the FTND.[8] Latent class analyses suggest that the FTND ranks smokers in a manner that corresponds fairly well to an empirically derived method.[19]

The HSI comprises only two items, which limits the relevance of internal consistency estimates. However, zero-order correlations between the two items in the measure indicate moderate levels of association (e.g., r's ≈ .30).[18] Both of these items tend to load statistically on a factor typically labeled "compulsive smoking."

The FTND and HSI predict both behavioral and biochemical indices of smoking (e.g., carbon monoxide [CO], cotinine, lifetime amount smoked).[4,5,7,14,20,21] This should not be surprising, given that the FTND and HSI directly assess smoking heaviness. However, it is encouraging to note that smokers are able to estimate their amount of smoking as indexed by biochemical tests in response to single items (e.g., "How many cigarettes/day do you smoke?"). The FTND has demonstrated an ability to predict cessation outcomes in smoking cessation studies.[18,22–25] However, the HSI appears to account for much of the predictive validity of the FTND.[5,18,26] Population-based studies conducted in Australia, Canada, the United Kingdom, and the United States found that the two HSI items (number of cigarettes smoked per day and time to first cigarette [TTFC] in the morning) were the strongest predictors of quitting.[27,28] Furthermore, later research has shown that a single item on both the FTND and HSI, the TTFC, predicts relapse vulnerability as well as, or better than, much longer multidimensional instruments.[18] Additional population-based research shows that a single item on the HSI (TTFC) is highly effective in predicting the likelihood of future cessation.[18] Finally, latent class analyses suggest that the TTFC is highly informative for discriminating empirically derived classes.[19]

The *DSM* and the *International Statistical Classification of Diseases and Related Health Problems*

Two different diagnostic systems commonly are used to diagnose tobacco dependence, and both typically are considered to be unidimensional measures of tobacco dependence. One is the *DSM-IV,*[1] which is based on an empirically driven, syndromal medical model, rather than on a theoretical model of dependence. The second is the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10),*[29] an international diagnostic classification system that came into use in World Health Organization member states in 1994. Most of the extant research has utilized *DSM* criteria and

***DSM-IV* Criteria**

1. Tolerance
2. Withdrawal
3. Use in larger amounts/over longer period than intended
4. Persistent desire/unsuccessful efforts to cut down or quit
5. Great deal of time using/recovering
6. Important activities given up
7. Continued use despite emotional/physical problems

HSI Questions[a]

1. "At present, how long after waking do you wait before having your first cigarette (in mins)?"
2. "How many cigarettes do you smoke per day at present?"

[a]HSI questions from Heatherton and colleagues.[5(p793)]

will be the focus of this chapter's review of diagnostic classifications of tobacco dependence.

Structured clinical interviews based on the *DSM* and the *ICD,* such as the World Mental Health Survey Initiative version of the Composite International Diagnostic Interview (CIDI)[30] or the National Institute of Mental Health Diagnostic Interview Schedule (DIS), comprise a series of branching questions that are aimed at eliciting information about features relevant to nicotine dependence; they have been translated into various languages and used in multiple population-based studies.[31–34]

Data on the reliability and structure of diagnostic interview measures of nicotine dependence arise from studies using face-to-face administration strategies. Therefore, the following conclusions cannot necessarily be generalized to a different administration format. There is evidence that the various structured diagnostic measures yield reliable diagnoses as assessed by test-retest reliability ($\kappa = .63$),[35] $\kappa = .88$,[33] and $\kappa = .73$.[36] One factor analysis indicated that responses to the CIDI had a strong single-factor structure,[37] although other factor analyses of the structured diagnostic items found that a two-factor structure was a better fit.[38–40]

Evidence suggests that the small set of dichotomous *DSM* items can distinguish between light versus heavy smoking.[37] An epidemiological study found that the *DSM* (third edition revised *[DSM-III-R]*), as assessed by the DIS, was a significant, though weak, predictor of cigarette abstinence over one year, but that the FTND was a better predictor, and that number of cigarettes smoked per day was the best predictor.[26] Another study showed that *DSM-IV* diagnoses of nicotine dependence predicted heaviness of use and cessation outcome in a population-based study of college students.[41] Several studies have shown that *DSM-IV* nicotine-dependence diagnosis is associated with greater risk of psychiatric comorbidities in adults and youth.[35,42,43] In sum, there is substantial evidence that *DSM* and *ICD* diagnoses are meaningfully related to smoking heaviness and psychiatric status.

Multidimensional Measures of Nicotine Dependence

Multidimensional measures offer some promise in elucidating the nature of dependence and in helping to refine the phenotype so as to foster more informative genetic mapping. Figure 3.2 shows a watershed model of how genetic influences may affect a complex phenotype across ontogeny.[44] This model conveys the notion that a final disease phenotype may be the product of diverse types of influences, and that some influences may be operative for some people, while other influences are operative for other people. However, these

diverse "feeder stream" influences are somewhat compensatory and interchangeable with respect to contributing to "downstream" processes that produce mature features of nicotine dependence. It is conceivable that these influences may exert additive or interactive effects and that they might be differentially sensitive to environmental events. Such influences could be viewed as reflecting the myriad influences that constitute quantitative trait loci.

The assumption is, however, that there is a "final common pathway" (ultimate downstream) set of processes and symptoms that is manifest once a disorder achieves some level of severity. Thus, at clinical levels of a disorder, sufferers appear similar to one another, but this similarity may mask diverse etiologic paths. Diagnostic measures of dependence such as the *DSM,* FTND, and HSI are intended to index the "final common pathway" of nicotine-dependence processes, rather than the "feeder streams" (relatively discrete pathways) that may individually and collectively influence the disorder and that (in theory) share stronger relations with particular genetically influenced biological processes. These measures can be labeled "distal" in that they are relatively remote from the genetic variants that the phenotypic measures are intended to reflect.

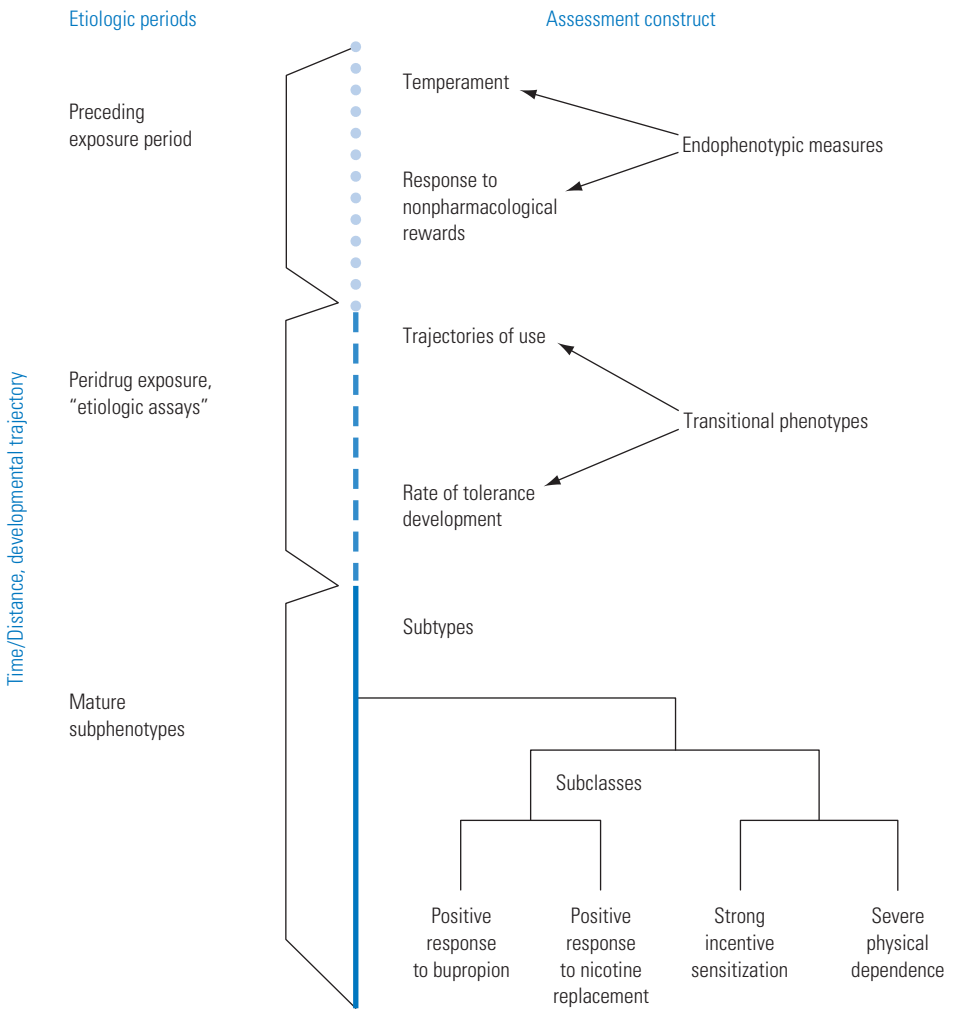
The FTND, HSI, and the *DSM*-type diagnostic measures were intended to measure a unitary, synthetic clinical manifestation of dependence (albeit, the measures may not in fact be unidimensional). The two distal measures reviewed below are intended to be multifactorial. They were developed in response to emerging data that nicotine dependence itself appears to comprise multiple dimensions.[45] The relevance of such measures to genetic mapping is that they contain heterogeneous items, which may help elucidate the sorts of items that are, and are not, sensitive to genetic variants (permit distillation of the phenotype). Further, if nicotine dependence involves multiple components, assessment of each dimension may permit the detection of subgroups of smokers who show unique or qualitatively different manifestations of dependence. Subgroups that differ on the basis of relevance or intensity of dependence dimensions are termed *mature subphenotypes* in this chapter. The concept of the "mature subphenotype" is based on the notion that some groups of smokers may differ qualitatively in dependence such that different measures of dependence are more sensitive to dependence in one subgroup versus another (figure 3.3). This would occur if the processes that contribute to the final common pathway of dependence (e.g., tolerance, tendency to relapse back to tobacco use, activation of incentive structures in response to nicotine anticipation) do not completely mask diversity in etiology.

Two relatively new multifactorial scales have been developed that are designed to identify somewhat distinct dimensions of nicotine dependence. These are reviewed here with an eye toward evaluating their basic features and their potential utility in genetics research. It is important to bear in mind that neither of the reviewed instruments was designed specifically to assess nicotine-dependence dimensions for the purpose of molecular genetics research; other goals were operative, such as isolating the relatively distinct motivational dimensions of dependence.

Nicotine Dependence Syndrome Scale

The Nicotine Dependence Syndrome Scale (NDSS) is a 19-item self-report measure developed to assess nicotine dependence on the basis of Edwards's (1976) theory of the alcohol dependence syndrome.[46] Edwards's theory identified the core elements of alcohol dependence as (1) narrowing of the repertoire of drinking behaviors, (2) increased salience of drink-seeking behaviors, (3) increased tolerance,

Figure 3.3 Etiologic Path and Locus of Phenotypic Assay



Note. Phenotypes may be dynamic (e.g., responses to environmental challenges such as drug dose, withdrawal). Some processes may exert effects across etiologic periods.

(4) occurrence of withdrawal symptoms, (5) use of alcohol to avoid or relieve withdrawal, (6) subjective awareness of a compulsion to drink, and (7) a tendency to resume alcohol use after abstinence.[47] The NDSS comprises five different subscales: Drive—craving, withdrawal and smoking compulsions; Priority—preference for smoking over other reinforcers; Tolerance—reduced sensitivity to the effects of smoking; Continuity—regularity of smoking rate across place and time; and Stereotypy—the invariance of smoking. The NDSS has the advantages of a clear theoretical basis and evidence[46] that shows that either the whole scale, or some individual subscales, are significantly related to nicotine-dependence indices such as smoking heaviness, withdrawal measures, and other dependence measures such as the FTND. In addition, the NDSS can distinguish between chippers (chronically light smokers)

and heavy smokers.[48] Thus, it would be possible to use these subscales to select subgroups or types of smokers or to relate genetic variants to a continuous dependence subdimension represented by these scales.

Although the NDSS has promise as a measure of nicotine-dependence subtypes, the scale could be improved further for genetics research for the following reasons: (1) Some individual subscales have modest internal consistencies (or reliabilities), which undercut their use in measuring discrete dependence subtypes or elements.[49] (2) Some subscales comprise quite disparate types of items. For example, the Drive subscale mostly comprises items that measure withdrawal, but not exclusively. The somewhat heterogeneous item sets were apparently needed because of the breadth of the constructs targeted. This item heterogeneity also accounts, no doubt, for the modest internal consistencies of some of the subscales. Differential weighting of items via factor scores does not apparently mitigate this effect.[49,50] (3) Like the other distal measures previously reviewed, the NDSS subscales do not appear to tap constructs that are tightly linked to relatively discrete, fundamental biological processes that should, themselves, reflect variation in the genetic variants of interest. Although the NDSS does not provide specific indices of particular biological processes, its subscales assess relatively discrete dimensions of nicotine dependence; these may shed light on the core features of dependence, which might, in turn, promote more effective assessment strategies.

Wisconsin Inventory of Smoking Dependence Motives

The Wisconsin Inventory of Smoking Dependence Motives (WISDM) is a 68-item scale that comprises 13 subscales.[50] The primary goal in developing the WISDM was to create a theory-based research instrument to identify fundamental motivational processes that ultimately influence dependence criteria (e.g., relapse, withdrawal severity). In other words, the scale is designed to measure motivational influences that lead to dependence features or criteria.

The WISDM comprises the subscales listed in table 3.1 (table 3.1 also provides a rationale for each subscale). The WISDM has some advantages for genetics research. One is that the overall scale score and many of the subscales predict classic dependence criteria such as self-administration rate, withdrawal magnitude, and relapse.[50] Moreover, each subscale has acceptable reliability. This means it may be used profitably as an independent assay of a particular smoking motive.

The subscales were designed to reflect discrete motives that drive tobacco use in addicted individuals. Some of these motives may be associated with particular biological response systems and structures that may suggest genes that deserve investigation. As an example of this, the Taste/Sensory Processes subscale was developed because research showed the importance of gustatory and sensory cues in motivating smoking.[51,52] Taste sensitivity, especially the ability to taste bitter flavors, is related to phenylthiocarbamide (PTC) haplotype status.[53] Subsequent research has shown that smokers who achieve higher scores on the Taste/Sensory Processes subscale tend to possess PTC haplotypes associated with an inability to taste bitter tastes.[54] In other words, those smokers who can taste bitter flavors are less likely to smoke for taste reasons. The importance of specificity in the assessment of dependence dimensions is suggested by the finding that the Taste/Sensory Processes subscale became more highly associated with PTC status once nontaste items were removed from the subscale. Thus, the relation depended on taste per se, rather than other orosensory factors.

Table 3.1 Subscales of the Wisconsin Inventory of Smoking Dependence Motives

Subscale	Construct rationale: evidence base
Affiliative Attachment	Use of addictive drugs, including nicotine, is motivated by the impact of the drug on social affection systems and is manifest as emotional attachment to the drug.[55,56]
Automaticity	Drug self-administration and supportive information processing becomes automated.[57,58]
Behavioral Choice/Melioration	Drug use is inversely proportional to constraints on access to drug and to other reinforcers.[59]
Cognitive Enhancement	Nicotine enhances cognitive processing or via suppression of withdrawal.[60] This may be especially important to certain populations.[61]
Craving	Craving reflects not only magnitude of physical dependence[50] but also error signals indicative of conflict over drug-use decisions in such structures as the anterior cingular cortex.[62]
Cue Exposure/Associative Processes	Conditioned responses to drug cues activate drug motivational processing and encourage self-administration and may reflect activity in dopaminergic incentive systems.[63]
Loss of Control	Strong dependence motivation is related to the perception of loss of volition.
Negative Reinforcement	Drug use is motivated by strong negative affect occurring via either withdrawal or stressors; source of negative affect may be linked with relevant processing substrata such as the amygdala or extended amygdala.[57]
Positive Reinforcement	Drug use is motivated by desire to experience mood enhancement (rush, high) even in the absence of distress; may be linked to mesotelecephalic structures such as the nucleus accumbens.[64,65]
Social/Environmental Goads	Social cues associated with drug use can increase drug motivational processing or self-administration.[55,66]
Taste/Sensory Processes	Taste and orosensory processes play a strong motivational role in smoking; may be linked to the phenylthiocarbamide haplotype and associated gustatory sensory systems.[51,52,67]
Tolerance	Rate of tobacco clearance and tolerance to nicotine actions may permit high levels of self-administration; may be linked to nicotine metabolism or distributional tolerance in the brain.[68,69]
Weight Control	Nicotine appears to lower body weight set-point, and this may motivate nicotine self-administration,[70] especially among those seeking weight loss; may be related to sensitivity to nicotine's effects on hypothalamic weight regulatory centers or to systems that affect taste hedonics.

Other subscales, such as Cue Exposure/Associative Processes and Positive Reinforcement, were designed to reflect activity in dopaminergic structures, such as the nucleus accumbens, that impart or mediate the processing of the incentive value of drug cues as well as drug induced pleasure or reward.[63,71–73] Such responses may account for the potent impact of drug-paired cues on nicotine motivation.[74]

Although the WISDM subscales hold some promise for reflecting relatively discrete dimensions of nicotine dependence, like the NDSS subscales, they also have significant limitations. For example, psychometric analysis has shown that some of the subscales are highly correlated with one another and load onto a common factor. In other words, these subscales may measure a final common pathway (figure 3.2) more than a discrete dependence motive. In addition, although an attempt was made to link the targeted discrete motives with underlying biology, for many of the subscales the self-report dimensions are,

no doubt, only remotely related to activity in any particular biological system. Finally, it seems clear that even with 13 subscales, there are potentially discriminable dimensions that should be assessed but are not. For example, one could easily argue that one useful subphenotype might be the anticipatory excitement or arousal that precedes drug use in a motivated, deprived smoker.[75] Another target might be the anhedonia of withdrawal—that is, the inability to experience pleasure during withdrawal, which may be related to elevated reward threshold in mesotelencephalic dopaminergic systems.[76] Finally, it is unclear that some of the individual subscales of either the NDSS or the WISDM share strong relations with classic dependence criteria (e.g., relapse).[46,50] Thus, the construct validity of each subscale must be demonstrated before strong inferences regarding nicotine dependence can be made (figure 3.1).

In summary, both multifactorial measures of nicotine dependence (i.e., the NDSS and the WISDM) have some promise for measuring relatively discrete dimensions of nicotine dependence, and these measures will, no doubt, prove useful as predictors of relapse, withdrawal, and other nicotine-dependence criteria. In addition, some particular subscales may have potential utility in molecular genetics research. However, some of the subscales are not ideal for this purpose. The constructs they target cannot be tightly related to an underlying biology, and some of the subscales appear to reflect broad, rather than specific, dimensions of nicotine dependence.

Smoking, Initiation of Smoking, and Distal Measures of Dependence

Distal measures have shown that nicotine dependence is under considerable genetic control. Heritability of *DSM-III-R* nicotine dependence was estimated to be 60% in a sample of Vietnam veteran male twins[77] and 44% in Minnesota adolescents.[78] Moreover, biometric modeling suggests an overlap (60%) in the genetic substrata for smoking versus the development of nicotine dependence[79] but also a moderate residual genetic effect for nicotine dependence (22%). In general, such modeling shows proportionally larger genetic contributions to nicotine dependence than to smoking or smoking initiation and smaller environmental influences.[80–86] Thus, evidence shows overlap in the genetic influences for initiation of smoking and the development of nicotine dependence, and genetic influence that is unique to dependence.[87,88] Accordingly, distal measures have the potential to identify nicotine-dependence phenotypes that do, and do not, have associations with causal genetic variants.

Epidemiological research using distal measures also has revealed that heavy smoking and nicotine dependence can be extremely common, at times almost modal, across large populations. Thus, it is possible, or even likely, that large portions of the population possess genes that promote or permit such phenotypes. Under such a circumstance, it may be a more viable strategy to search for genetic influences that discourage or prevent regular tobacco use, rather than to identify the potentially ubiquitous variants that permit nicotine dependence; that is, variants that discourage nicotine dependence might have greater discriminative efficiency. There is precedent for this in the alcohol literature in which polymorphisms of the *ALDH2*2* and *ADH1*2* alleles appear to affect alcohol metabolism. A proposed mechanism of influence for these alleles is that they code for increased levels of the metabolite acetaldehyde, which may impart unpleasant peripheral effects

that discourage high levels of alcohol intake.[89,90*]

Distal measures have also revealed another important general feature of nicotine dependence: it is not equivalent to regular smoking per se. Indeed, epidemiological data show that a significant proportion of daily smokers, perhaps one-half, do not warrant nicotine-dependence diagnoses.[32,91] Thus, research shows that many individuals may engage in heavy amounts of smoking and yet never report having experienced strong withdrawal, that their smoking is out of control, or that they have given up important activities because of their smoking (i.e., with dependence indexed by *DSM* or *ICD-10* type of criteria). These observations are consistent with the notion that there appear to be degrees of severity in nicotine dependence even among inveterate smokers, at least to the extent that commonly used distal measures have some validity as measures of nicotine dependence. The researcher's task is to determine how to measure dependence in a manner that reflects its biological and genetic influences.

In summary, distal measures have revealed that (1) nicotine dependence is under considerable genetic control, (2) it is equivalent to neither regular smoking nor smoking initiation, and (3) its genetic origins are somewhat distinct from those that support or permit the development of regular smoking. These observations suggest that while regular smoking may be a component of nicotine dependence, nicotine dependence assays must go beyond assessments of smoking features per se to capture important dimensions of the construct.

A Core Dimension of Nicotine Dependence

One of the anomalies in dependence assessment is that although dependence measures often show poor internal consistency and poor relations with one another,[33,49] factor analyses show that such measures often load highly onto common factors; even when the factor analyses suggest multiple factors, the factors are highly intercorrelated.[46,50,92] In addition, zero-order correlations often show strong interrelations among particular dependence measures.[18,50]

When items derived from the FTND, HSI, or diagnostic criteria are factor analyzed, they show that measures that tap into heaviness of smoking, and pervasiveness of smoking across time or occasion, tend to load most highly on principal component or initial factors. For instance, Muthén and Asparouhov[39] factor analyzed items tapping *DSM-IV*[1] symptoms of dependence in a general population sample. This research showed that symptoms were best accounted for by a multidimensional model. The pattern of covariation among the symptoms yielded a first factor with relatively high loadings for items assessing "tolerance," "larger amounts," and "time spent using." "Tolerance" reflects taking increased amounts of nicotine/tobacco to achieve desired effects. "Larger amounts" reflects self-administering nicotine in larger amounts, or over longer periods of time than intended. "Time spent using" reflects the amount of time the individual expends in actual smoking, procuring cigarettes, and so on. Thus, the first factor seems to be highly related to the amount smoked and the amount of time spent smoking. The second

*Of course, protection versus vulnerability is a relative thing; one haplotype could be viewed as a vulnerability factor, or its complement could be considered a protective factor. However, it may be possible to show both protective and vulnerability effects versus a "neutral" haplotype. More to the point, the search for protective factors (versus vulnerabilities) might suggest different phenotypes, different genetic variants, different measurement cut-scores, and different experimental designs.

Inferences about Dependence Derived from Distal Measures

Many distal measures of nicotine dependence have modest psychometric properties (i.e., reliability and validity). For example, scales such as the Fagerström Tolerance Questionnaire (FTND)[a] and scales comprising *Diagnostic and Statistical Manual of Mental Disorders (DSM)* items tend to have modest internal consistencies (scores on the items are not highly correlated with one another).[b] There is also copious evidence that some dependence scales (e.g., the FTND and the *DSM* criteria) are not highly correlated with each other.[b,c,d] A number of factors could account for this lack of agreement—for instance, measures or items that are poorly worded, a lack of variance in terms of the assessed construct in the sampled populations, error that differentially affects the measures, and the fact that the different assessments are measuring somewhat different constructs. Curiously, the lack of agreement of dependence measures is actually quite useful. It allows one to determine which types of measures are highly related to each other, and to dependence criteria, and which are not. This provides some insight into core features of dependence and permits the distillation of essential features. Data are considered in this chapter that link particular measures of dependence to principal dependence criteria (e.g., as depicted in figure 3.1).

It appears that the evidence of agreement or commonality among dependence measures is much greater than the evidence of disagreement or inconsistency. Moreover, the evidence of disagreement can be accounted for by logical distinctions among dependence constructs that are targeted by measures and by the fact that different measures or items are susceptible or vulnerable to different sources of error.

[a]Heatherton, T. F., L. T. Kozlowski, R. C. Frecker, W. Rickert, and J. Robinson. 1989. Measuring the heaviness of smoking: Using self-reported time to the first cigarette of the day and number of cigarettes smoked per day. *British Journal of Addiction* 84 (7): 791–99.

[b]Piper, M. E., D. E. McCarthy, and T. B. Baker. 2006. Assessing tobacco dependence: A guide to measure evaluation and selection. *Nicotine & Tobacco Research* 8 (3): 339–51.

[c]Breslau, N., and E. O. Johnson. 2000. Predicting smoking cessation and major depression in nicotine-dependent smokers. *American Journal of Public Health* 90 (7): 1122–27.

[d]Moolchan, E. T., A. Radzius, D. H. Epstein, G. Uhl, D. A. Gorelick, J. L. Cadet, and J. E. Henningfield. 2002. The Fagerström Test for Nicotine Dependence and the Diagnostic Interview Schedule: Do they diagnose the same smokers? *Addictive Behaviors* 27 (1): 101–13.

factor was somewhat more related to "persistent desired/unsuccessful efforts to cut down or quit," and "continued use despite emotional/physical problems."[39(p1052)] Confidence in this solution is bolstered by the fact that it was obtained in three separate, relatively large groups of individuals (*N*s = 8,552–26,946). Thus, the three types of items that loaded onto the first factor reflect heaviness or consistency of use across time.

Other factor analytic studies have generated complementary patterns of findings. Lessov and colleagues[92] constructed biometric models with a sample comprising male and female dizygotic and monozygotic twins (as well as different-gender dizygotic twins) who all said that they had either experimented with, or tried, smoking (*N* = 6,249). As part of this research, the authors factor analyzed individual nicotine-dependence items obtained from the *DSM-IV* dependence criteria as well as the two items that constitute the HSI derived from the FTND.[5] The authors reported a two-factor solution. The first factor consisted of the two HSI items (TTFC and cigarettes smoked per day [CPD]) and the *DSM-IV* Tolerance item (largest number of cigarettes smoked in a single day). The second factor consisted of DSM items concerning withdrawal, smoking more than intended, experiencing difficulty

quitting, and smoking despite physical or psychological problems. Again, the first factor extracted reflects the heaviness and pervasiveness of smoking. It is notable that the TTFC item loaded more highly on the first factor than on the second factor, upon which the withdrawal item loaded. This suggests that the TTFC item reflects a pattern of heavy smoking rather than severe withdrawal after overnight abstinence. These results are consistent with results obtained when FTND items are factor analyzed (results discussed earlier). That is, the principal component of such items is consistent with a pattern of heavy, pervasive smoking, and the TTFC item tends to load on this first factor.[8,13] Research using multidimensional scales sheds additional light on these findings (discussed below).

Other research using distal instruments has yielded similar findings, with items reflecting heavy use constituting an initial factor explaining the majority of variance in the set of items or instruments,[38] while secondary factors reflect variance related to withdrawal severity, instrumental reasons for smoking (e.g., to suppress withdrawal), or an inability to quit. Although there is some variability in the results of such factor analyses (e.g., the principal axis and maximum likelihood factor analytic solutions in Breteler and colleagues[13]), the bulk of research suggests that most of the variance in diagnostic criteria and the FTND is captured by items that reflect a pattern of heavy smoking.

There are various reasons that such a pattern of results might not be interesting or important. For instance, it may be that items reflecting smoking heaviness have the greatest representation on the factor-analyzed[93] instruments, and this accounts for their high loadings on the initial factors. Or, it may be that items asking about smoking heaviness or pervasiveness are simply easier for smokers to answer than are other items, and therefore, they can be answered with relatively little error. This might occur because such items have some fairly discrete referents (e.g., number of cigarettes smoked per day, time of day of initial smoking). However, while relative saturation of true score variance may account for relatively high levels of covariance among such items, such an effect, by itself, could not account for the substantial evidence that items that tap smoking pervasiveness and heaviness have impressively strong and consistent relations with some critical dependence criteria and also appear to reflect dispositions that are highly heritable.

The two items making up the HSI, in particular, have shown impressive relations with a host of behavioral and biochemical measures that reflect smoking heaviness.[4,5,7,26,93] Interestingly, these items have also been more consistently predictive of the ability to quit smoking than perhaps any other set of dependence measures.[18] For instance, in a Transdisciplinary Tobacco Use Research Center (TTURC) paper,[18] the TTFC item in the FTND was shown to be superior to multiple alternative measures in predicting the likelihood of successful cessation. In fact, this item showed greater predictive validity than any instrument with which it was compared (e.g., the NDSS and its subscales, the WISDM and its subscales, and the FTND total score and any other single item from that scale). Interestingly, the TTFC item predicted relapse vulnerability better than did biochemical measures of smoke exposure such as CO, suggesting that it may reflect behavioral and motivational components of self-administration not entirely captured by drug dose delivered per se. These findings on the TTFC are impressive in that they were demonstrated in multiple clinical samples. In addition, this item was shown to predict cessation likelihood in population samples gathered in four different countries.[18] While there is substantial evidence that the TTFC item is consistently predictive of quitting likelihood, there is also substantial evidence that *both* items of the HSI have impressive predictive

validities, relative to other instruments, for both cessation likelihood and indices of tobacco consumption.[3,7,8,26]

There is also substantial evidence that measures of smoking heaviness are highly heritable. For instance, in a study by Lessov and colleagues,[92] items that loaded most highly on the first factor (*DSM-IV* Tolerance, TTFC, and CPD) had somewhat higher heritability estimates (additive genetic effects: variance components = .68–.73 as estimated via the *univariate* model) than did the other items. In addition, a later paper by Haberstick and colleagues estimated heritability coefficients for the FTND, the HSI, and individual items on those scales.[94] The sample comprised 1,154 young adults between 18 and 25 years of age who were from full-sibling, half-sibling, and twin pairs. Multivariate modeling revealed a highly heritable factor (76%), the strongest salient of which was time to first cigarette in the morning (i.e., TTFC). The HSI also generated a substantial heritability estimate (61%). These estimates agree with those generated by other studies.[88] Haberstick and colleagues[94] conclude that the TTFC item assesses an "urgency" to smoke throughout the day and is the "single best measure in the FTND for examining the genetic contributions to nicotine dependence."[94(p663)]

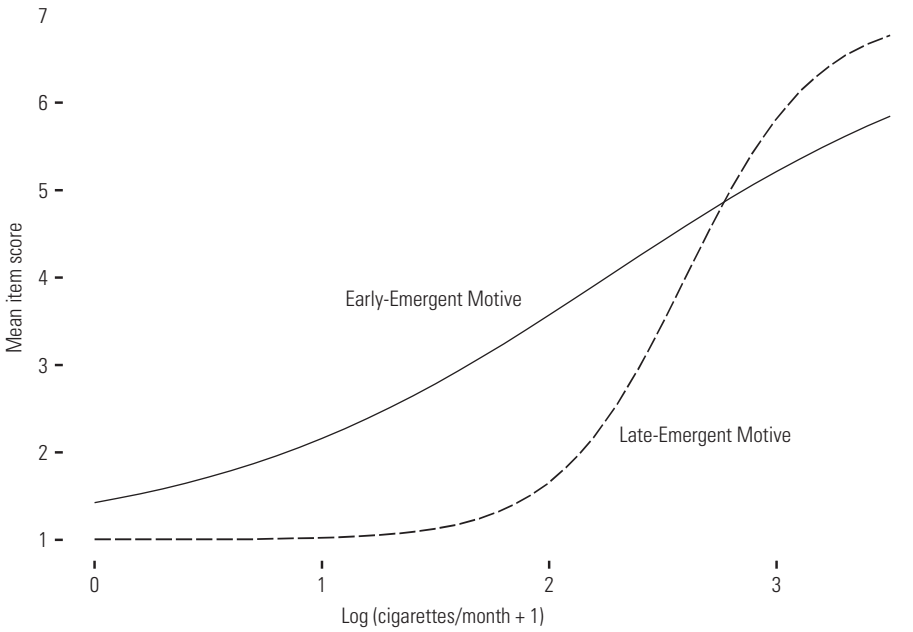
Therefore, the evidence suggests that items that tap heavy and pervasive smoking—for instance, smoking that begins perforce as soon as the individual wakens—assess a core feature of nicotine dependence, one that is highly heritable. This conclusion is buttressed by additional research, reviewed below, that uses the newly developed multifactorial measures of nicotine dependence. Thus, these measures appear to be serving one of the functions for which they were designed—that is, elucidating the nature of dependence.

As noted previously, the TTURC paper[18] showed that the TTFC item was significantly related to both cigarettes smoked per day and to relapse likelihood. In addition, this research showed that this item was highly correlated with a small number of subscales from the WISDM and NDSS nicotine dependence questionnaires. In particular, the TTFC was related to the Tolerance and Automaticity subscales from the WISDM and the Stereotypy subscale from the NDSS. These subscales appear to assess a pattern of smoking that is heavy and pervasive (fairly continuous across time and context) and that has become highly ingrained or automatic (i.e., does not involve conscious cognitive control).

It is interesting that the scales so highly correlated with the TTFC item are those that measure characteristic "late-emergent" dependence motives[50]; that is, light smokers are relatively less likely to endorse these motives, relative to other sorts of motives (e.g., smoking for taste, smoking in response to environmental cues) than are heavy smokers. Figure 3.4 depicts the different logit curves reflecting scores on two WISDM subscales, Tolerance and Social/Environmental Goads, relative to cigarettes smoked per month. (The term *late emergent* refers to appearance across the continuum of smoking heaviness; these data may not reflect the order of emergence across time, because the data are cross-sectional and do not permit strong inferences about developmental patterns.) It is clear that the Tolerance subscale is relatively insensitive to light amounts of smoking but that scores increase exponentially at high smoking rates. (The Automaticity subscale showed a similar ogive pattern.[50]) This suggests that items that tap a pervasive smoking pattern or tendency are particularly sensitive to high levels of smoking.

Latent profile modeling with the WISDM suggests that smoking that is heavy, pervasive, and automatic may be both necessary and sufficient for significant nicotine dependence. Across four separate samples of smokers, the WISDM

Figure 3.4 Logistic Regression Curves Predicting Scores on the WISDM Subscales from Cigarettes Smoked per Month: Examples of an Early-Emergent Motive (Social/Environmental Goads) and a Late-Emergent Motive (Tolerance)

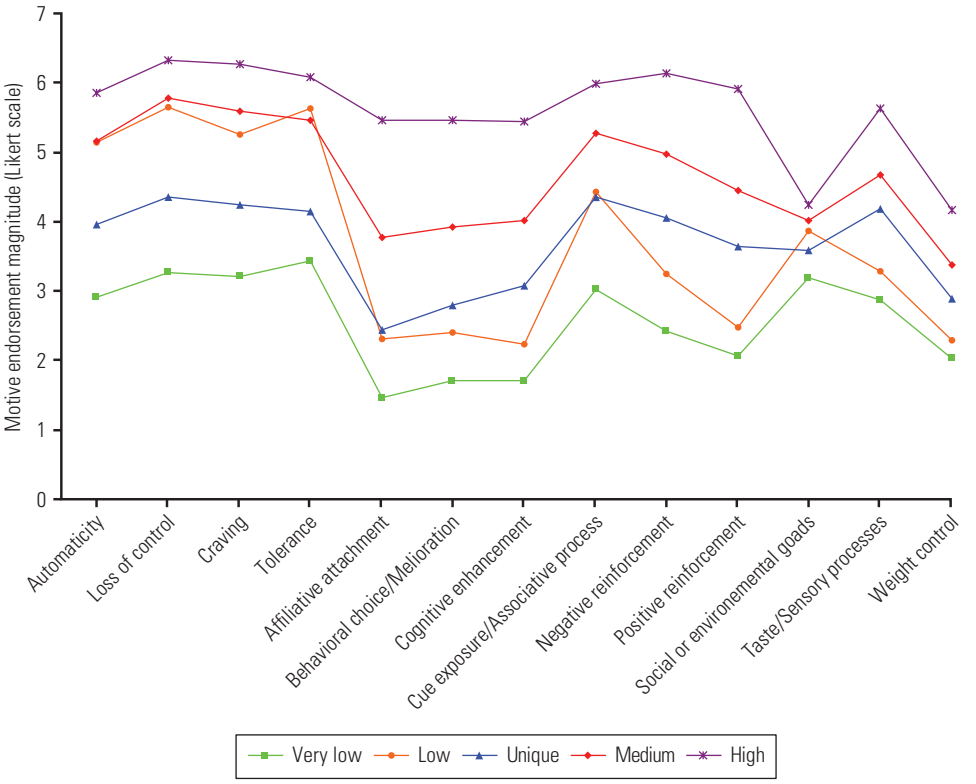


Note. This figure illustrates the different curves of the early-emergent motives and late-emergent motives, using the Social/Environmental Goads and Tolerance scales as prototypes of each motive, respectively. The early emergent motive has a higher intercept at low rates of smoking than does the late-emergent motive and has consistent linear growth as smoking rates increase. The late-emergent motive is not endorsed by light smokers but as smoking rates increase, there is an exponential increase in the rate of endorsement. WISDM = Wisconsin Inventory of Smoking Dependence Motives.

Automaticity, Tolerance, Craving, and Loss of Control subscales (table 3.1) characterized a unique smoker profile.[95] Some smokers had high scores on only these four subscales (figure 3.5). All other smokers showed subscale elevations that were of relatively equal magnitude across the subscale types. The results show that no group of smokers was significantly dependent without having elevations on these four subscales. Piper and colleagues,[95] therefore, labeled these subscales as "primary smoking motives scales."

The latent profile analysis discussed above constitutes a person-centered analysis that highlights subscales that may be necessary for significant dependence development. However, these results, by themselves, do not flesh out the construct validity of the amalgam of these subscales as a synthetic assay of nicotine dependence. To do this, Piper and colleagues[95] conducted variable-centered analyses in which status on the four primary motives scales was related to meaningful indices of nicotine dependence (see Muthén[96] for a discussion of the blending of person- and variable-centered approaches). The ability of these scales to predict these dependence criteria was compared to the predictive validities of the other WISDM subscales (labeled the "secondary motives" scales). Specifically, the relative predictive validities of each set of subscales were established.[95] It was then determined whether the secondary dependence motives

Figure 3.5 Latent Class Results from the Combined Data Set



Note. The profiles generated via latent profile analyses of some 2,256 smokers from different data sets that contained both treatment seekers and general smoker populations. The same basic profile patterns were present in all four samples of smokers when they were analyzed separately.

could account for significant variance in dependence criteria once the primary motives scales were entered into regression models.

These variable-centered analyses indicate that a limited subset of WISDM subscales, the primary motives scales, carry the lion's share of predictive validity regarding nicotine dependence. The primary dependence motives scales (Automaticity, Craving, Loss of Control, and Tolerance), by themselves, were highly predictive of such important dependence criteria as ability to maintain abstinence, scores on other dependence measures (i.e., the FTND), smoking heaviness (i.e., cigarettes smoked per day, baseline CO), smoking history (i.e., age of initiation, age of daily smoking, number of previous quit attempts), and the magnitude of the increase in craving that occurred immediately postquit.[95] The relative validity of these scales versus the secondary scales can be gauged from analyses in which the mean score for the primary dependence motives was entered into prediction models along with the mean score for secondary motives. Such analyses revealed that the predictive validity of the primary dependence motives scales was little affected by the addition of the secondary scale composite in the models. To an impressive degree, the primary scales remained consistently predictive of the dependence criteria in the multivariate

models, while the predictive relations of the secondary scales became weak or anomalous (negatively related to criteria). Finally, it is important to note that these four primary motives scales are highly coherent, with an average intercorrelation of about $r = 0.77$. Ironically, the multidimensional instruments might have contributed to the overall understanding of dependence by illuminating the "final common pathway" of dependence, rather than by assessing subphenotypes.

The notion that heavy, automatic smoking is indicative of dependence fits with other data in the field and is in accord with the view that, as dependence becomes entrenched, control over smoking is shifted from cognitive-control systems to automatic motor-control systems that execute self-administration without such control and, perhaps, without awareness.[18,58,62] Thus, as smoking becomes ubiquitous and automatic, smokers may believe that it has become noncontingent with instrumental uses.[13,97,98] Considerable basic behavioral and neuropharmacological research supports the notion that dependence involves a shift from instrumental, goal-driven behavior to automatized, habitual response patterns. As Everitt and Robbins[99] note in an influential review in 2005:

> In theoretical terms, it seems reasonable to characterize such compulsive behavior as a maladaptive stimulus-response habit in which the ultimate goal of the behavior has been devalued so that the behavior is not directly under the control of the goal…. Rather, responding is governed by a succession of discriminative stimuli, which also function—when they are presented as a consequence of instrumental responses—as conditioned reinforcers. Hypothetically, such stimulus-response associative ('habit') learning occurs in parallel with instrumental action-outcome learning but, with extended training, eventually dominates behavioral output.[99(p1485)]

Thus, smokers with this unique profile may represent highly dependent individuals in whom this process is more advanced or who are simply more aware of its occurrence (and therefore, rate secondary motives relatively low).

The Craving subscale of the WISDM was identified as one of the primary dependence motives. This is compatible with the notion that as addictive behavior becomes automatic, urges are caused by blockade of the automatized drug self-administration sequence.[58,62] That is, the co-occurrence of strong craving and high levels of automaticity is supported by theory that links the two constructs mechanistically.

Another source of evidence further buttresses the notion that a pervasive pattern of heavy smoking indexes dependence. A study by Goedeker and Tiffany[100] used taxometric procedures to determine whether nicotine dependence constitutes a taxon (best conceptualized as a category of individuals qualitatively different from other individuals) or a continuum in which individuals lie on a relatively continuous range. The authors used data from the 2003 National Survey on Drug Use and Health ($N = 11,441$) and employed multiple criteria to assess the structure of dependence. The results supported the notion that nicotine dependence can be viewed as a taxon—a qualitatively discrete category. Approximately 48% of those smoking in the last 30 days belonged to this taxon, and members of this taxon were characterized by high scores on the FTND TTFC question (smoking relatively soon after awakening), by smoking a large number of cigarettes per day, and by high scores on three of the NDSS subscales: Drive, Continuity, and Tolerance. Drive taps craving intensity; Continuity taps smoking patterns that are consistent over time—that is, patterns that show little variation due to situational or temporal factors; and Tolerance assesses the tendency

or ability to smoke heavily without adverse impact. Thus, the taxon appears to be distinguished by measures that tap the same sorts of constructs that characterize the necessary and sufficient features of dependence as revealed in the latent profile research (i.e., the primary dependence motives)[95] and that are effective at predicting relapse.[18,95] Thus, the hypothesized taxon is characterized by smoking that is heavy and pervasive throughout the day and by strong urges.

Additional evidence also supports the fundamental relation between a pervasive drug-use pattern and dependence. For example, Shiffman and Paty[101] found that chippers (those who have established a stable pattern of infrequent smoking) and heavy smokers are distinguished by the fact that the former evidence smoking that is contextually discriminated, whereas the latter show patterns that are relatively heavy and invariant across time and place. In addition, as noted earlier, examination of posterior probabilities generated by latent class analyses show that items that assess pervasive, heavy smoking tend to distinguish classes that are highest in dependence.[34,39,102]

The reviewed research suggests that the highly dependent person is *not* best distinguished by endorsements of smoking as a means of controlling affect, reducing withdrawal, experiencing a "high," or controlling weight.[18,95] This perspective meshes nicely with a great deal of behavioral animal research that shows that early in the course of drug self-administration the organism's behavior is highly affected by the potency of the reinforcer. However, with extensive drug self-administration experience, the animal's behavior seems more stimulus driven and noncontingent with the reinforcer.[103,104] The data from the latent profile study[95] suggest that some smokers may become aware of this noncontingency and can report on it.

The data from the TTFC paper,[18] the taxometric paper,[100] and the latent profile studies[95] all suggest that dependence is characterized by smoking that is not highly discriminated on contextual and temporal cues. One possibility is that truly dependent smoking takes on a life of its own and proceeds without cueing. This, however, would fly in the face of a great deal of evidence that shows that cues can powerfully affect self-administration and other indices of drug motivation,[57] and it would contradict the notion that addictive behavior reflects strong stimulus-response mapping.[104] Instead, it seems much more likely that smoking is highly cue dependent, but that the cueing is often relatively inaccessible to awareness. This might occur because the cues are interoceptive (e.g., reflective of falling levels of drug in the body), or are exteroceptive, but the cue–self-administration response sequence has become proceduralized and unfolds with little awareness.[57,58,62]

One of the roles of dependence assessments is to provide insight into the nature of dependence processes—insights that extend beyond those afforded by the direct assessment of dependence criteria per se[49] (figure 3.1). The review of the evidence presented above suggests that the use of multidimensional dependence scales may be achieving this goal by casting in greater relief those behaviors and motives that are most tightly linked to dependence criteria. It is clear that the distal measures implicated in dependence in the above research (e.g., the Automaticity and Tolerance subscales) do not truly assess underlying dependence processes per se, but they may serve as manifestations of such processes—for instance, implicating mechanisms such as the strengthening of stimulus-response mapping.

The above analysis suggests that existing distal measures that are especially sensitive to pervasive, automatic, and heavy

smoking probably tap processes of greater biological significance and relevance to dependence than do measures that tap social or functional consequences of smoking or an awareness of such consequences. Therefore, measures such as the FTND that more uniformly tap pervasive, heavy smoking (and presumably related scales from multidimensional instruments)[97,98] should yield stronger and more informative genetic relations than does the collection of *DSM* criteria. Evidence from behavioral and molecular genetics studies is beginning to support this hypothesis.[92,94,105–107]

Covariation Among Measures of Dependence

If it is indeed the case that nicotine dependence can be assessed reliably by a relatively coherent set of items, this does not explain the lack of consistent covariation among dependence measures that was noted above.[26,33,49,98] This lack of covariation is likely to be caused by several factors. First, the various dependence indices were developed with guidance from very different conceptual models of dependence (see West[3]), and as the construct validation model makes clear (figure 3.1), different conceptual models will generate very different types of items. For instance, the model that guided the development of the *DSM* measures defined "dependence" as a socially defined phenomenon indexed by a collection of indicants that, together, reflect severity. It is an implicit assumption of this model that the features should not necessarily be highly coherent in that they are intended to convey additive, and not necessarily redundant, information that indexes extent of behavioral, functional, and social disruption. Thus, such measures were designed, in part, to reflect awareness of diverse types of social and functional disruption, which could be viewed as *criteria,* or socially important outcomes, of dependence, rather than dependence processes per se (figure 3.1). One reason that criteria may have modest relations with measures of dependence mechanisms (e.g., selected subscales of the NDSS or WISDM) is that measures of social or functional disruption are highly dependent upon the social and life context of individuals and the functional demands placed upon them. And, interestingly, as West[3] points out, the diagnostic items used in the *DSM* and other major diagnostic inventories were designed originally to diagnose other types of addictive disorders in which the drug leads to greater social and functional impairment. Thus, these items may be of limited use in assessing mechanisms of tobacco dependence.[3] This means that very different sources of error and extraneous influences likely affect criterion measures than affect core dependence measures—for example, patterns, types, and intensities of smoking.

The same principle applies to other criteria such as relapse. It should be of no surprise that there are inconsistent or modest relations between dependence measures and relapse likelihood in that relapse likelihood is strongly related to such variables as whether smoking is permitted in the home, the educational and income status of the individual, and the density of smoking cues in the person's environment.[18,108–111] Similarly, dependence measures often show modest relations with withdrawal severity.[112–114] Withdrawal severity has been shown to reflect such environmental features as the presence of smoking cues.[115] Many criteria measures are highly sensitive to contextual influences but are also necessary to make inferences about the construct validity of any dependence measure (figure 3.1). This raises questions about how to distill variance within criteria so they are maximally sensitive to biological or genetic influences and how to model dependence in the face of modest intercorrelations among the criteria.

There are other reasons that dependence measures may show modest relations

with one another. For instance, some measures may be more sensitive to dependence at different periods or intensities in its development.[49,116,117] This is consistent with evidence that dependence items show different patterns of endorsement across different latent classes that are organized along an intensity dimension;[102,118–120] that is, some types of items are more sensitive to low versus high levels of dependence, and other items are more sensitive to severe dependence. Thus, disagreement might be attributed to differences in the "difficulty level" of an item.[12]

Integrating Phenotypic Measures with Analytic Strategies

Selecting good measures of the phenotype is just one step in examining the relation between the phenotype and genetic variants (single nucleotide polymorphisms, alleles). One must decide how to use such measures so they sensitively capture differences among individuals in terms of nicotine dependence. This demands an integration of both theoretical and psychometric considerations.[12]

One strategy often used in genetics research is the construction or selection of groups that are intended to be maximally dissimilar in possession of targeted genetic variants. Ideally, one attempts to construct groups on the basis of phenotypic features, so one group has all the genetic influences that promote a disorder, while the other group has none (an "extreme" groups approach).

The information reviewed above suggests strategies that might be used to construct such groups. For example, it suggests that a group possessing the genetic complement for nicotine dependence should show high scores on the scales and items that reflect heavy, pervasive, automatic smoking (table 3.2). A critical question is whether the investigator needs to use additional criteria to determine membership in this group. For instance, the investigator must decide whether to make membership or nonmembership contingent upon factors such as additional dependence dimensions (in addition to the primary dependence features discussed above), the presence of person factors associated with type or severity of dependence (psychiatric comorbidity, gender), and factors that

Table 3.2 Dimensions on Which Groups Might Be Constructed to Contrast Putative High- and Low-Dependence Predispositions

High genetic proneness	Low genetic proneness
▪ Smokes within 30 minutes of awakening ▪ Lifetime peak smoking >20 CPD ▪ Severe withdrawal upon reducing or quitting smoking ▪ Reports great difficulty in quitting/failure to quit in multiple attempts ▪ Daily smoker for at least 20 years ▪ High scores on WISDM subscales: Loss of Control, Automaticity, Tolerance, Craving ▪ High scores on NDSS subscales: Tolerance, Continuity, Drive	▪ Smokes after 30 minutes of awakening ▪ Lifetime peak smoking <15 CPD ▪ Mild or no withdrawal upon reducing or quitting smoking ▪ Reports ease of quitting in few attempts/successful long-term abstinence ▪ Initiated smoking before the age of 16 years ▪ Smoked daily for at least 1 year ▪ Currently a nonsmoker for at least 2 years ▪ Low scores on WISDM subscales: Loss of Control, Automaticity, Tolerance, Craving ▪ Low scores on NDSS subscales: Tolerance, Continuity, Drive

Note. CPD = cigarettes smoked per day; WISDM = Wisconsin Inventory of Smoking Dependence Motives; NDSS = Nicotine Dependence Syndrome Scale.

might moderate the relation of genetic variants with phenotypes.

Assessment of Complementary Dimensions of Dependence

Considerable evidence attests to the centrality of the primary smoking factors that have been highlighted (pervasive, automatic, and heavy smoking). In keeping with this, it may be beneficial to supplement the measures listed in table 3.2 with some additional measures that tap the same construct to achieve a more reliable index of this central construct. Thus, one might use biochemical measures of self-administration (serum cotinine levels), metabolic tolerance or clearance,[121] and perhaps, laboratory measures of the automaticity of information processing related to self-administration and related constructs.[58]

In addition, there may be some value in including other dependence characteristics to supplement the assays of primary factors. Ideally, one would wish to select assessments that are associated with fairly severe dependence and that are fairly highly heritable. Further, it seems best to select assays that are not highly infused with error. The considerations listed above suggest at least two types of measures that might be combined with measures of the *primary* or *core factors* to yield groups extreme across the breadth of the nicotine-dependence measurement domain—namely, measures that tap difficulty or inability to cut down or quit (control) smoking and severity of withdrawal symptoms (e.g., related *DSM*-type items). Including assessments of withdrawal and ability to cut down or stop among phenotypic measures is supported by three considerations: (1) Items tapping these factors are conceptually and psychometrically distinct from measures of smoking heaviness and pervasiveness (core features). For instance, they tend to load on different factors than do items measuring the primary factors.[39,92] In fact, the evidence is compelling that while measures of smoking heaviness and pervasiveness do account for variance in withdrawal severity and quitting ability, much of the variance in these criteria is orthogonal to heaviness indices.[46,49] If these dependence criteria are critical to the construct, they should be reflected in group composition. (2) Items tapping ability to quit or cut down and tapping withdrawal severity tend to show high levels of endorsement by the most dependent smokers as revealed by latent class analyses.[39,92,102] (3) At least with regard to global ratings of withdrawal intensity, there is evidence of only partial overlap with the heritability of items that measure the primary dependence factors.[34,122,123] Therefore, the inclusion of such criteria for extreme group membership (i.e., ratings of ability to cut down or quit and withdrawal measures) might permit a more comprehensive gleaning of dependence-relevant genetic variants. The addition of measures of withdrawal and ability to cut down or stop smoking means that the criteria for extreme group membership would assess the five nicotine-dependence symptoms that Lessov and colleagues[92] found had "high phenotypic and genetic factor loadings as well as high heritability: tolerance, time to first cigarette in the morning, number of cigarettes smoked per day, withdrawal, and difficulty quitting."[92(p875)] Moreover, items tapping these dimensions would correspond to the dimensions that Furberg and colleagues[124] identified as distinguishing latent classes of regular cigarette smokers: smoking heaviness and latency to smoke upon awakening, difficulty or inability to cut down or quit smoking, and severity of withdrawal symptoms.

Consideration of Person Factors

It is clear that smokers are a heterogeneous group. Moreover, some individual differences or person factors (stable traits), other than nicotine dependence per se, might reflect

a different type or severity of nicotine dependence. Do such differences have implications for genetic mapping? Might such factors be relevant to assessing the nicotine-dependence phenotype or suggest particular genetic targets for association with phenotypes? For example, phenotype measures could be modified to target the assessment of such person factors. Or, a researcher could take such person factors into account when constructing extreme groups. Thus, questions relating to person factors really speak to the notion of whether such factors organize types of nicotine dependence.

It is tempting to imagine that psychiatric comorbidity may reflect affective or motivational processes that create protean complexity in the nature or structure of nicotine dependence. There is certainly evidence that comorbidity affects the manifestation of nicotine dependence (hence its influence on nicotine dependence as depicted in figure 3.1). For example, nicotine dependence is highly comorbid with other psychiatric disorders. Rates of current alcohol abuse or dependence, mood disorder, anxiety disorder, or personality disorder are two to three times more prevalent among smokers than among nonsmokers.[35,43] Not only are psychiatric comorbidities more common among smokers, but also smoking and nicotine dependence are also especially prevalent among those with such comorbidities.[35,43,125,126] Thus, a person with a psychiatric disorder is much more likely to have nicotine dependence, and vice versa.

In addition, some data suggest that the presence of comorbidity not only indexes an increased likelihood of nicotine dependence but also a more severe form. For example, data show that smokers with psychiatric comorbidities smoke a disproportionately large number of cigarettes given their prevalence in the population,[35] suggesting heavier smoking among those with comorbidities. Further, analytic strategies such as latent class analysis show that the presence of comorbidities helps define the classes that generate the most extreme scores on nicotine-dependence assays.[21,35,43,102,127] Finally, there is evidence of substantial shared genetic influence on the regular use of tobacco and alcohol and on dependence on both substances.[77,128,129]

Despite all the evidence linking externalizing disorders with nicotine dependence, there is little evidence that nicotine-dependence assessments, or construction of extreme groups, should be modified on the basis of comorbidity. Moreover, there are reasons for assuming that while comorbidity is associated with dependence severity, it does not index a qualitatively distinct subtype of dependence. In other words, even if such comorbidity affects the severity of dependence, this effect is captured by standard dependence assays. This is suggested by the studies showing that standard dependence measures are sensitive to comorbidity-linked increases in dependence.[35,102] In addition, as noted earlier, some evidence suggests that personality dimensions associated with comorbidity are more tightly linked with smoking *initiation* than with severity of nicotine dependence.[60,82,130,131] Thus, personality could affect exposure to environmental factors related to initiation (e.g., peers).[132–137] Finally, individuals with no comorbidity become nicotine dependent. The goal of phenotypic refinement in the pursuit of genetic correlates dictates that investigators focus on phenotypic features that are necessary or sufficient, and psychiatric comorbidity is neither.

If psychiatric comorbidities are not intrinsic to dependence and do not organize meaningful nicotine-dependence taxa, what might explain the associations between comorbidity and nicotine-dependence measures? The available evidence makes various causal relations possible. It is possible that a personality dimension

such as behavioral undercontrol, which is associated with externalizing disorders and traits of risk-taking and impulsivity, increases the likelihood of initiating the use of diverse substances. Such traits may also increase exposure to environmental factors such as availability, modeling, and other peer group influences. Such environmental influences could account for why comorbidities seem to be associated with a particularly severe form of nicotine dependence.[21,35,43,102,127] That is, the genetic diathesis for personality and/or comorbidity would influence initiation of nicotine use and, in addition, might yield environmental exposures that foster greater nicotine use over the lifetime (e.g., poor educational achievement, socializing with smoking or substance-using peers); hence, an active gene-environment correlation may be at work. It is, of course, possible that while psychopathology and associated personality dimensions do not produce different types of nicotine dependence, other factors do. For instance, there is evidence that gender interacts with nicotine-use motivation such that men tend to smoke more for nicotine receipt per se but women smoke more for nonpharmacological factors.[138] It may be that taste and correlated environmental factors have been rendered more reinforcing in women because of nicotine's ability to modulate incentive value.[139] Thus, it is possible that, among men, dependence phenotypes are more highly related to genes that influence direct pharmacological reinforcement. Among women, dependence may be more highly related to genes that influence incentive sensitization and related associative processes. At present, there is insufficient evidence to determine the relevance of such evidence for genetic mapping.[92] Similarly, there is insufficient evidence, at present, to support tailored assessment on the basis of race.[140,141] In sum, as opposed to the case of a disorder such as schizophrenia, for which there is some consensus that the diagnostic category comprises multiple distinct disorders, each perhaps with unique genetic influences, it does not appear necessary at this point to try to target specific subtypes of severe nicotine dependence.

Preserving a Role for the Environment

Earlier, this chapter alluded to the important role of the environment in creating error in measures of nicotine dependence. For example, living in a home that has a smoking ban may cause one to smoke later in the day than would otherwise occur. This may bias TTFC items as measures of nicotine dependence.[18] It is possible that such home smoking bans might ultimately reduce dependence, but this is merely a hypothesis, one that might require some time to unfold. Another example is smoking restrictions at work, which might reduce the number of cigarettes per day that individuals can smoke. Indeed, increasing environmental restrictions on smoking may affect the validity of inferences based upon all of the measures of heavy, pervasive, uniform, and automatic smoking that undergird the central core of available measures of nicotine dependence. In addition, the presence of smoking cues and cigarette availability might also lead to a greater likelihood of relapse to smoking in any given quit attempt. Researchers may take possible environmental influences into account in several ways. For instance, the investigator might use environmental features when constituting extreme groups, ensuring that members of both low- and high-dependence groups have few environmental restrictions on their smoking. Or, the researcher could use statistical procedures to perform partial variance in dependence assays. These options are discussed further below.

Environmental influences may play an additional role. Increasing attention is being directed at the notion that genetic variants may interact with environmental events or characteristics.[142] Indeed, there

is evidence that such interactions may be relatively common. Moffitt and colleagues[143] list multiple examples in which the relation of genotype with the phenotype varied significantly as a function of some environmental factor.

In considering whether to pursue investigation of potential gene-environment interactions, it is important to consider whether there are good candidate environmental factors that significantly affect the disorder under study. Moffitt and colleagues[143] suggest that good candidates for environmental moderators, or risk factors (i.e., "environmental pathogens"), are variables that exert significant main effects on the disease severity or occurrence. This principle suggests numerous environmental factors that might moderate gene-environment interactions—for example, early exposure to smoking peers, chronic environmental stress/poverty, intrauterine exposure to nicotine, traumatic stress such as childhood or adolescent sexual assault/abuse, and alcohol intake.[60,142,144] Thus, intrauterine, developmental, and adult-onset events, both episodic and chronic, could serve as pathogens.

One candidate moderator of particular interest is age of onset of significant nicotine exposure. There is substantial evidence for a sensitive developmental period after which tobacco exposure is relatively unlikely to yield nicotine dependence. Human research shows that an early onset of smoking is associated with greater consumption of cigarettes in adulthood,[145–148] a relative inability to quit smoking,[28,145,149,150] and a more severe form of nicotine dependence.[34,102,151–153] Animal research shows that nicotine exposure during adolescence induces long-lasting biochemical, anatomical, physiological, and behavioral changes that differ markedly from those seen with adult exposure.[154–157] In addition, adolescence is both a period of heightened sensitivity to nicotine's rewarding actions and a period of decreased sensitivity to nicotine's aversive actions.[158–162] Evidence suggests that adolescent exposure in rats results in increased nicotine self-administration that persists into adulthood.[163] Therefore, it is possible that genetic variants for nicotine dependence will become much more strongly associated with nicotine-dependence phenotypes among those individuals with early, rather than late, exposure (perhaps smoking before versus after 16 years of age).[34] In fact, later research shows a significant interaction between haplotypes of the *CHRNA5-A3-B4* subunit cluster and age of smoking onset, such that there are strong associations between haplotypes and a rather comprehensive set of dependence measures, including the FTND, WISDM, and relapse latency.[164,165]

It is possible that a variable might not produce a significant main effect on nicotine-dependence measures but could still produce significant moderation (e.g., if the pathogen were active in the presence of a relatively rare allele/genotype). Therefore, one should also assess and test pathogens that are substantively important. For example, some theories emphasize the importance of stress as an important modulator of reinforcement via psychomotor stimulants,[166,167] and other theories emphasize the role of peers, especially in terms of initiation.[60] In short, selection of candidate environmental variables in moderation models requires an examination of empirical evidence as well as theory.

Of course, moderated analyses include gene-pathogen relations as well as pathogen-phenotype relations. That is, one must decide not only which pathogen(s) to explore, but also which genetic variants would, in theory, affect behavioral or biological processes that are differentially affected by the pathogen. Therefore, one should have a model of the gene-pathogen relation that makes biological sense. Thus,

one advantage of testing moderated relations is that it forces one to think deeply about the potential causal links between genes, pathogens, and the phenotype.

Moffitt and colleagues[143] suggest a set of considerations to guide the selection of genetic variants (e.g., haplotypes, alleles) that might serve as good, independent variables in moderational models. For example, they note that candidate polymorphic variants should occur relatively frequently in the population. The notion is that if a gene exerted a powerful *main* effect on a significant disease process, its frequency would be suppressed because of decreased fitness (although it is unclear if risk for smoking would significantly decrease fitness). In addition, selection would be aided by evidence that the polymorphism has effects on brain systems relevant to a disorder and that it affects reactivity to the environmental pathogen/event under study. Thus, in the case of nicotine-dependence research, investigators using early exposure to tobacco as the environmental pathogen might study polymorphisms that are related to nicotine self-administration in animal research (chapter 4). Finally, the specificity of a particular gene-pathogen-disorder relation can be tested by systematic substitution of different polymorphisms and environmental pathogens into the analyses.

The study of moderation is compatible with the study of relatively specific mature subphenotypes. For example, moderated relations may pertain to only a subset of those with nicotine dependence; that is, only a subpopulation *should* be affected by the targeted gene as a function of the environmental pathogen* (see table 3.3).

Thus far, researchers have not detected stable gene-environment interactions in molecular genetics investigations of nicotine dependence. There have been reports of an interaction between status on the serotonin transporter gene *(SLC6A4)* and neuroticism in the prediction of likelihood of being a smoker.[168,169] However, as Lerman and colleagues[169] suggest, the interaction may be attributed to distinguishing between more and less highly heritable forms of neuroticism, or it may reflect epistatic effects. (See Kremer and colleagues[170] and Gerra and colleagues[171] for further research on *5-HTTLPR* status and smoking.)

Findings of interactions would pose interpretive challenges.[143] For example, environmental exposures may appear to "cause" a phenotypic response, but the environmental exposure may occur because it is correlated with genes and therefore indexes only a third (genetic) variable effect. For example, reports of severe stress may reflect the presence of polymorphisms related to neuroticism. This possibility could be appraised by careful assessment of the phenotype before and after the occurrence of the stress or stressor.

There are other challenges to the evaluation of gene-environment interactions; principal among them is the appropriate measurement and modeling of the pathogen. Recall measures of environmental pathogens may be biased by a host of memory/recall

*Here, the reader may justifiably ask what is meant by a smoker "subpopulation" or mature subphenotype. This does not *necessarily* imply the existence of multiple taxa, that is, fundamentally different types of smokers in terms of dependence. Rather, the researcher might find that some smokers may differ qualitatively or quantitatively in the extent to which certain processes contribute or relate to dependence. However, these differences do not mean that core features or manifestations of dependence differ. For instance, smokers may differ in the extent to which they smoke for taste factors. However, despite this motivational difference, dependence per se could still be registered by standard dependence measures. Thus, smokers may differ in taste as a motive, and this may have a distinct genetic basis,[54] but the dependence of such smokers might still be captured well by the major distal phenotypic measures.

Table 3.3 Causal Paths from Genetic Variant to Distal Phenotypes

	Nicotine reward	Metabolic capacity	Taste/gustatory sensitivity	Incentive salience/ sensitization	Cognitive control/ impulsivity	Withdrawal	Affective control
Genetic variant candidates	*CHRNB2* *CHRNA7* *CHRNA5*	*CYP2D6* *CYP2A6* *CYP1A1*	*PTC*	*DRD2* *SLC6A3* *DRD4*	*MAOA*/α4 subunit	α7 subunit/ $GABA_A$	SLC6A4
Endophenotypic index candidates	Proteomic/ expression patterns	Proteomic/ expression patterns	Inability to taste bitter tastes	Enhanced nucleus accumbens activity to nondrug incentives	a. Behavioral Undercontrol b. P300 amplitude c. Poor Stroop test performance d. Attentional focus on dominant response e. Poor integration of caudal cingulate with amygdala (via fMRI)	Information processing performance after removal of an appetitive stimulus	a. Poor fear/anger extinction b. Internalizing symptoms upon stress exposure c. Poor subgenual cingulate-amygdala integration (via fMRI) d. Stronger urges in response to stress
Transitional/mature subphenotypes	a. Report of nicotine reward ("buzz," "rush") b. Rapid escalation of smoking upon initiation c. High self-administration rates	a. Rapid development of tolerance b. High self-administration rates	a. Taste motive for smoking	a. Strong nucleus accumbens response to nicotine anticipation	a. Rapid escalation of smoking upon initiation b. Externalizing comorbidity	Severe withdrawal symptoms upon drug abstinence	a. Relapse in response to stressors b. Smoking for stress relief
Distal phenotypes	a. Heavy, regular self-administration b. Withdrawal symptoms upon abstinence c. Tendency to relapse						

Note. fMRI = functional magnetic resonance imaging.

biases, and convenient self-report measures may not capture important temporal dynamics of such variables over time. In addition, one must decide at what age or developmental period the pathogen is most active. Furthermore, one must consider the latency between pathogen occurrence and its impact on the phenotype—addictive behavior tends to be relatively refractory over many years. This must be considered when trying to model the time course via which a pathogen might affect nicotine-dependence markers and at what etiologic stages such effects would be manifest. Although relapse latency might be reactive to a phasic environmental event, other markers of nicotine dependence might be relatively refractory. Moreover, Moffitt and colleagues[143] note that some pathogens exert cumulative effects[172,173]—for example, living with a smoker. In sum, modeling the potential effects of a pathogen on nicotine dependence requires consideration of developmental period, etiologic period, the dose of pathogen needed to exert effects, the latency between pathogen exposure and disease end points, and which particular phenotypic features will be affected by the pathogen.

Improving Distal Measures

Earlier, this chapter reviewed evidence suggesting that existing distal measures have considerable potential in genetic mapping research. However, it is important that investigators be aware of the limitations of these measures. Such limitations not only should foster caution in drawing strong inferences regarding underlying mechanisms but should also serve as prods for the development of new phenotypic assessment strategies.

Although the reviewed data suggest some phenotype measures to use in future genetics research, restricting phenotypic measures to *DSM* and FTND items could significantly handicap researchers. For example, use of a single item to measure withdrawal severity captures an impoverished domain of targeted constructs (e.g., *DSM*-type withdrawal items do not assess different types of withdrawal symptoms, such as urges and hunger). Also, different withdrawal symptoms show very different profiles or trajectories over time.[174] It is unlikely that global, temporally remote, single-item, self-report measures of withdrawal can capture the distinct dynamic patterns of withdrawal over time—patterns that account for differential likelihood of relapse.[115] Further, at present, all of the commonly used distal measures rely upon self-report, and this assessment strategy may be influenced by broad attitudes and verbal resemblances that have little to do with the biological or genetic underpinnings of dependence. Moreover, diagnostic criteria, and similar sorts of categorical items, were not designed to possess optimal scale properties or to covary highly to achieve internal consistency. In addition, if used as a categorical diagnostic index, the *DSM* generates outcomes that do not agree with empirically based methods of nicotine-dependence classification such as latent class analysis. For example, a factor mixture analysis[39] did not correspond well with the scoring rules for dependence as specified in the *DSM-IV* (i.e., three of seven dependence symptoms must be endorsed). Many individuals placed in the highest class of dependence via factor mixture analysis did not satisfy the *DSM* threshold for dependence. (See Storr and colleagues[19] with respect to correspondence between latent class analysis and FTND classifications of smokers.)

Thus, commonly used nicotine-dependence measures have clear limitations, which, in theory, should limit their effective use in genetic mapping. There are clearly opportunities for new approaches to dependence assessment. Obviously, one approach would be to develop superior measures of the same constructs.

For instance, it is by no means clear that the wording and structure of existing items are ideal for genetics research. Future research should attempt to improve upon the existing dependence items by examining issues such as whether their response options are ideal and whether the questions are posed at the proper "difficulty level." It is also possible that some of the newer multidimensional dependence instruments might be psychometrically superior to the critical *DSM* and FTND items and assess the same or similar constructs.

Another strategy would be to make greater use of behavioral measures. For instance, one strategy would be to relate genetic variants to withdrawal data recorded via ecological momentary assessment methods or to laboratory measures such as compensatory behavior in response to nicotine restriction. These strategies seem to offer clear advantages. Behavioral measures tend to be face valid (their significance requires little inference), can be measured with precision, and should be relatively free of certain self-report biases (e.g., response styles).

Although the direct assessment of behavior or criteria has potential, it is not yet known whether this approach will be superior or inferior to the collection of general, synthetic, and impressionistic measures of withdrawal, or ability to quit smoking, and so on. For instance, measures of a single episode of withdrawal might overweight the idiosyncratic events affecting that quit attempt episode. Thus, the more impressionistic, global measure might provide a better synthesis of withdrawal across time and a better index of the attendant subjective distress. In addition, some evidence suggests that some temporally remote assessments of smoking and dependence can be surprisingly accurate and reliable.[175,176] Thus, while complex behavioral or laboratory measures hold promise, they remain undelivered promissory notes. Perhaps the optimal behavioral assays of nicotine dependence would require collection of data over long periods of time, permitting the statistical synthesis of data gathered across repeated episodes of cessation attempts and withdrawal. This would also permit extensive behavioral assessment of the heaviness or situational pervasiveness of smoking (e.g., time blocks per day in which no smoking occurs, average intercigarette interval, extent to which smoking is cue contingent), perhaps via ecological momentary assessment.[177]

Concerns about the sensitivity of phenotypic measures to underlying biology anticipate issues to be addressed in later sections of this chapter that deal with the assessments of intermediate phenotypes—that is, more focal and specific measures of nicotine dependence.

A Summary of Inferences from Distal Measures Research

The picture of nicotine dependence emerging from the reviewed research is reflected in a fairly coherent set of core features: smoking is heavy and pervasive across time and place, occurs without significant cognitive control, and is related to strong urges. Although such features appear to constitute core, relatively coherent elements, dependence also seems to be reflected in somewhat distinct complementary factors. In particular, withdrawal severity and an inability to quit or cut down on nicotine or tobacco use seem to be important in this regard. Certainly, other constructs are related to dependence and predict dependence criteria. For instance, smoking for taste reasons (e.g., as assessed by the WISDM Taste/Sensory Processes subscale) is significantly related to dependence measures and to the likelihood of smoking.[54] Yet, research does not suggest that such factors are necessary or sufficient for severe dependence.[54,95]

Such assessments may, in fact, reflect "upstream" vulnerabilities to initiate or escalate smoking (figure 3.2), but they do not strongly determine variance in "downstream" levels of severity.

New Directions for Phenotypic Research: Beyond Distal Measures

The measures discussed above are all "distal" measures—that is, measures reflecting dependence indices that are likely causally remote from the biological processes activated by relevant genetic variants (figure 3.2). As figure 3.2 makes clear, mature dependence phenotypes may, as implied by the developmental concept of equifinality, constitute one or more rather homogeneous outcomes of diverse etiologic paths. The use of distal measures may limit and distort the appraisal of nicotine dependence for a variety of reasons.

It is possible that many of the conclusions adopted in the earlier discussion are largely a product of the measures available to us. For instance, the evidence that nicotine dependence can be well modeled by a dimension of heavy, pervasive smoking may be due, in part, to the global and categorical nature of distal measures. If measures are employed that reflect specific candidate biological pathways to nicotine dependence, it is possible that our view of nicotine dependence might change. For example, as figure 3.2 depicts, there may be diverse genetically influenced factors that promote and permit tobacco use, and these ultimately summate to yield nicotine dependence. It is conceivable that different specific (ontogenetic) pathways are more meaningful for some individuals than for others. For example, one person's heavy smoking might be driven by rapid nicotine metabolism, whereas another person's might be driven by strong dopaminergic response in brain incentive structures. "Downstream," or distal, measures might not be sensitive to differences in such discrete pathways. An often used analogy is the case of a car (or clock) that will not run. General distal measures (e.g., lack of motion, lack of exhaust) will provide superficial evidence that something is wrong. However, more focused measures that are sensitive to particular mechanical pathways are needed to detect specific causal mechanisms. Thus, it may be that phenotypic measures targeted at specific biological pathways are needed to produce a group of smokers who share (are homogeneous for) a particular genetically mediated vulnerability to nicotine dependence. In theory, multidimensional dependence assessments such as the NDSS or WISDM might serve this need. However, such measures may inadequately target particular biological systems. Indeed, an exclusive reliance on self-report may preclude precise targeting. Finally, it may be the case that comprehensive characterization of the phenotype requires a developmental research strategy; that is, a richer portrayal of the phenotype may emerge from measures gathered across ontogeny (figures 3.2 and 3.3).

The above reasons, and others, encourage the use of phenotypic measures that are sensitive to specific causal pathways thought to lead to nicotine dependence—pathways that are, in theory, more proximal to the biological effects of the polymorphisms under study and that are used in a developmentally informed manner. Dissatisfaction with global distal measures, and the desire to assess genetic risk across the development of disorders, have led to the assessment of intermediate phenotypes, or endophenotypes (see below).

A basic assumption of the endophenotypic approach is that genetic influences

will be more straightforward, and less complex, when the phenotype is relatively circumscribed (e.g., involving relatively few biological systems or processes), which should clarify the contributing genetic architecture. In addition, the endophenotype should be manifest relatively early in the causal chain leading to the syndrome of interest (it should be causally "upstream"; figure 3.2). The latter feature should enhance penetrance and, therefore, result in a stronger genetic signal. At the end of this section, some potential risks of the endophenotypic approach are reviewed. It is conceivable that mapping distal phenotypes could, under some circumstances, constitute a more efficient research strategy.

It is clear that genes do not encode for psychopathological syndromes or symptoms per se; however, they do encode for less complex biological and behavioral processes. Diseases or syndromes that affect multiple, diverse organ systems seem more likely to be affected by numerous causal influences, including genetic influences. Moreover, it also seems likely that as the number of genetic influences increases, so does the possibility of heterogeneity *across persons* in such influences. Measuring a distal outcome such as number of cigarettes smoked per day might obscure individual variation in phenotypic differences and genetic influences.

In short, using proximal phenotypes should enhance genetic mapping by tapping genetic signals with greater penetrance and by reducing multiple phenocopies.[178] An example of the advantage of relatively discrete phenotypic measures, and ones that are more tightly linked to underlying biology, can be found in the area of gene mapping in hypertension: stronger gene mapping was found for angiotensin-converting enzyme than for blood pressure or hypertension diagnosis.[179] Figure 3.2[44] conveys the notion that a final disease phenotype may be the product of diverse types of influences, and that some influences may be operative for some people, while other influences are operative for other people. However, these diverse "feeder stream" influences are somewhat compensatory and interchangeable with respect to contributing to "downstream" processes that produce mature features of nicotine dependence. It is conceivable that these influences may exert additive or interactive effects and be differentially sensitive to environmental events. The assumption is, however, that there is a final common pathway (ultimate downstream) set of processes and symptoms that is manifest once a disorder achieves some level of severity. Thus, at clinical levels of a disorder, sufferers appear similar to one another, but this similarity may mask diverse etiologic paths.

Endophenotypes, or "intermediate" phenotypes, link disease-promoting or disease-permitting sequence variations in genes to lower-level biological processes and link lower-level biological processes to the downstream observable syndromes that constitute diagnostic categories of disorders.[180,181] A genetic variant that affects an upstream process affects all the downstream processes that depend on it (figure 3.2). Thus, the genetic variants associated with a particular endophenotype should be associated with multiple (downstream) phenotypes that are causally dependent on that endophenotype. There may be considerable individual variation in the sets of possible upstream influences that contribute to nicotine dependence in one individual compared with another; that is, there is a final common pathway of processes, but highly heterogeneous influences may lead to that pathway. The next section discusses the concepts of "endophenotypes" and "transitional phenotypes" that differ from distal phenotypes in their specificity and causal priority.

Phenotypes along the Causal Chain of Dependence Development

In this chapter, the term *endophenotypes* is defined in a manner that is consistent with earlier definitions by Gottesman and others:[44,181,182]

- Endophenotypes should be heritable. The endophenotype cannot transmit information about genetic differences if it is not sensitive to such differences. Although endophenotypes must be heritable, they may also be highly responsive to environmental manipulations.
- Endophenotypes should be associated with the causes, rather than the effects, of disorders. Ideally, endophenotypes should be located in the causal path to the disorder, not be a consequence of the disorder or its treatment. Endophenotypes may also be useful to the extent that they are markers of a disorder (i.e., they correlate meaningfully with phenotypes that are on the causal path). A causal role for an endophenotype is suggested by the endophenotype preceding the disorder ontogenetically or developmentally in affected individuals (figure 3.3). Moreover, it should not appear to be merely a prodromal or less intense manifestation of the disorder.
- Assuming the endophenotype is heritable, the presence or magnitude of the endophenotype should reflect the genetic relatedness to an individual diagnosed or affected by the disorder. Thus, if appetitive motivational response to an anticipated reward is a heritable endophenotype for nicotine dependence, then two individuals of biologically similar relatedness to a smoker should show the same level of this phenotype, even if these individuals are discordant for smoking. However, this situation may not hold even with useful endophenotypes: the endophenotype may be heritable, and may precede the appearance of a disorder, but may also be affected by environmental factors and by the disease process per se. For example, some schizophrenics show abnormalities of the prefrontal cortex and working memory, and these deficits predate schizophrenia onset, consistent with an endophenotype. However, the evidence suggests that these deficits are also exacerbated by the illness,[44] showing apparent reciprocal relations between the endophenotype and disorder (or perhaps its treatment or other consequences of the disorder). A similar complex relation has been found for hippocampal volume differences, with such volume differences being related to genetic load for schizophrenia in a stepwise manner, and yet, the genetic load for schizophrenia appears to render a person more susceptible to the effects of fetal hypoxia on hippocampal volume. In other words, genetic load appears to *moderate* the effects of hypoxia on hippocampal volume. Reverse causation of this nature[183] may certainly occur in nicotine dependence; that is, ingestion of nicotine may affect phenotypic assays. This calls for careful separation of endophenotypes and *transitional phenotypes* (discussed below).
- The endophenotype should not be redundant with disorder status; it should account for only a portion of the variance in disorder severity or course. The endophenotype is intended to reflect more discrete features or risk factors for a disorder than are reflected in the disorder per se. Because of this, multiple endophenotypes would need to be identified to account for significant variance in a disorder phenotype and to identify genetic variants that confer risk.
- The endophenotype is a mediator. The molecular genetic variant should account for significant variance in

the endophenotypic measure, and the endophenotypic measure should account for significant variance in the phenotype. Ultimately, one would need to use multimediator models to explain large proportions of variance in the phenotype. This means that one needs two theoretical models: one of the gene-endophenotype relation and one of an endophenotype-phenotype (disorder) relation. Successful use of the endophenotypic approach requires that investigators test causal models comprising (1) specific biologically relevant processes thought to contribute to clinically meaningful nicotine dependence (the endophenotype-phenotype model); (2) specific genetic variants thought to influence those biological processes (the gene-endophenotype model); and (3) an assessment plan that is sensitive to the specific biological process(es) and to the ultimate phenotype.

- Endophenotypes should be present before, or in the absence of, a disorder (figure 3.3). Thus, as noted earlier, the endophenotype truly conveys a risk factor and not a prodromal feature or disease manifestation. Thus, to permit the strongest causal inferences, endophenotypes should be assessed before the development of nicotine dependence and be present in nondependent individuals as a function of consanguinity. Thus, endophenotypes are both more specific than the clinical phenotype and possess temporal and causal priority. However, it may be useful to distinguish a host of factors significantly more specific than the clinical phenotype but that manifest only when disease causal processes have been induced. These might be termed *transitional phenotypes* (see figure 3.3). Unlike endophenotypes, these do not occur before exposure to a pathogen (e.g., high stress, nicotine). Tolerance development to nicotine would be an example of such a transitional phenotype. Tolerance development could not be assessed directly outside of exposure to nicotine. Many of the important theories of nicotine dependence development really refer to phenomena that could be captured as transitional phenotypes. For example, the development of tolerance, withdrawal symptoms, sensitization to nicotine's incentive effects, and the development of conditioned reinforcement would all depend on exposure to the direct effects of nicotine. The distinction between endophenotypes and transitional phenotypes is an important one because very different causal claims are being made in the two cases. The distinction also has clear implications for experimental design: in the case of endophenotypes, one would assay, ideally, individuals with little or no nicotine exposure; in the case of transitional phenotypes, one would assay those who have initiated tobacco use.
- Endophenotypes should manifest causal effects across different levels of analysis or different points of the causal chain (table 3.4). Figure 3.6 shows a model in which an attention deficit endophenotype affects the likelihood of downstream transitional and mature phenotypes. In theory, the same polymorphisms might be related to phenotypic variance at all three stages of the causal path, to the extent that the endophenotypes were genetically influenced and served as setting events or instigators for the subsequent transitional and mature phenotypes. Thus, pleiotropy would be evident in these relations to the extent that an endophenotypic or transitional effect determined downstream manifestations of nicotine dependence. Of course, it is always possible that a phenotype that is associated with a developmentally early stage of smoking will not account for significant variance in measures of clinical, or mature, levels

Figure 3.6 Associating Genes with Phenotypic Stages

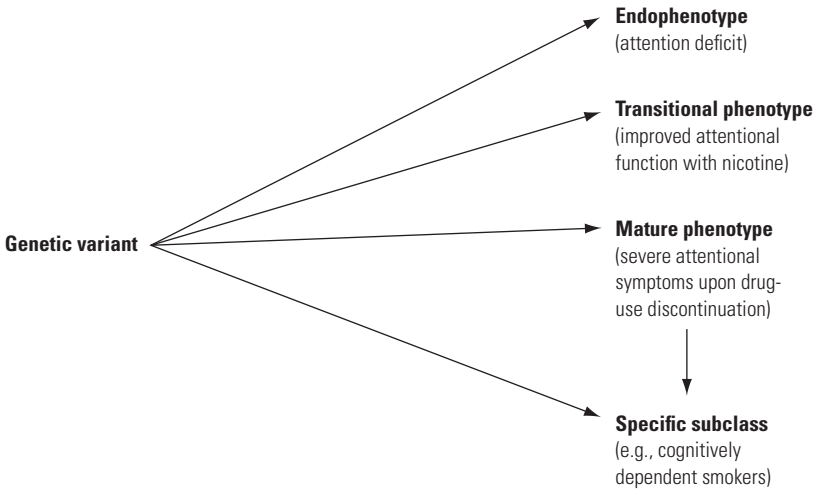


Table 3.4 Levels of Analysis in Characterizing the Phenotype

Level	Example
Transcripts/gene expression	Polypeptide synthesis
Postreceptor processing	Second-messenger response
Neurotransmitter system status	Response to nicotine challenge
Brain morphology	Density of receptor cell types
Physiological morphology and function	Liver clearance rates
Brain function	fMRI
Discrete cognitive processing	Anterior attentional function
Discrete behavioral domains	Smoking rate
Emergent cognitive function	IQ, memory
Emergent behavior/ psychological traits	Personality, attitudes

Note. fMRI = functional magnetic resonance imaging.

of dependence. Such factors might be endophenotypes for initiation rather than dependence.

- Figure 3.3 makes another factor clear: it is likely that different genetic variants are associated with risk at the different levels of etiologic development. For example, some genetic variants may contribute to nicotine-dependence development only after considerable nicotine exposure (e.g., variants that foster tolerance or permit heavy nicotine use). Thus, as figure 3.3 illustrates, a complete theoretical model of nicotine dependence should address which biological processes, associated with which genetic variants, influence nicotine-dependence development at which points in the causal path. The ability to track a postulated causal path across levels of analysis and across points in the causal pathway adds greatly to the strength of inferences linking all elements in the phenotypic causal chain.
- Endophenotypes for one disorder should predict occurrence of genetically related disorders. Some disorders are

substantially correlated with one another, and their intercorrelation is due to shared genetic influences.

Caveats to Assessing Endophenotypes

Although there are clear potential advantages to a strategy that focuses on specific endophenotypes, potential hazards exist as well. That is, it is possible that greater progress might be made, and made more quickly, by concentrating on mapping distal, or clinical, phenotypes rather than endophenotypes. This might occur because the endophenotype may itself be a nonspecific or promiscuous causal influence, giving rise to myriad outcomes (e.g., not only nicotine dependence but also a variety of externalizing and attention deficit disorders). Thus, the probability of a criterion given a particular response is not equivalent to the probability of a response given the criterion ($P [C \mid R] \neq P [R \mid C]$). In this case, one might detect strong associations between an endophenotype and genetic variants, but the relation might have little to do with nicotine dependence per se. In addition, it is unavoidable that individuals with nicotine dependence will possess numerous candidate endophenotypes. It is clear that the distal phenotype (at least with a large representative sample) comprises all of the possible endophenotypes and their genetic substrata. It is only in this context that the relative and incremental value of the relevant genes can be determined. That is, the endophenotype per se will likely not provide a context that allows one to identify those genetic variants that are *relatively* influential. Thus, the value of a particular genetic variant becomes known only after its relation with the distal phenotype is known. It may be most efficient to try to ascertain this relation directly without recourse to the endophenotype.

Another concern is that some endophenotypes may be very difficult to assess in a manner that is reliable and appropriate to the purpose of molecular genetics. For example, some potential endophenotypes (e.g., impulsivity) are very difficult to measure for any purpose and, in fact, may be more difficult to measure than is the distal nicotine-dependence phenotype. Related to this point, it is likely that only a portion of variance in an endophenotypic measure is, in fact, related to progression to a disorder end point. The task of refining endophenotypic measures appropriately could be tremendously difficult and time-consuming. Finally, it is important to remember that nicotine dependence, like other psychiatric disorders, is contextually and socially defined. A distal phenotype tells which individuals have developed a disorder despite, or because of, a host of environmental or developmental events. Different phenotypes (i.e., endophenotypes versus distal phenotypes) will reflect the influences of an entirely different set of developmental and environmental events. Thus, distal clinical measures may be most likely to reflect those genetic influences that create signal against a context of relevant environmental and life events. Of course, one could eventually discover and address these threats via endophenotypic research, but this might not be the most efficient strategy.

Finally, as noted earlier, there may be great value in studying specific subtypes among those in whom nicotine dependence is clearly present (*mature subphenotypes;* figure 3.3). These would, in theory, share the same virtue of specificity, as would endophenotypes and transitional phenotypes; that is, their greater specificity might permit stronger relations in genetic mapping than would occur with broader, more encompassing phenotypes. An example of a mature subphenotype might be smokers who are fast nicotine metabolizers or who smoke for taste reasons.[54,68] The use of mature subphenotypes need not depend on the assumption that

nicotine dependence comprises multiple unique taxa. The approach might be profitable even if one assumes that the disorder is affected by distinct, continuously distributed dimensions (neuroticism, behavioral undercontrol, reward reactivity, taste sensitivity, and so on). The strategy might reveal populations of smokers for whom certain dependence processes are relatively important.

Causal Paths Comprising Endophenotypic, Transitional, and Distal Phenotypic Measures

Although questionnaires hold promise for elucidating the genetic basis of nicotine dependence, a better understanding of the molecular genetics of nicotine dependence may require the use of additional assessment strategies (e.g., imaging strategies;[184] see chapters 7 and 8).

A tremendous variety of endophenotypic and transitional phenotypic assessments are potentially available. However, many are possibly quite costly and labor-intensive. In such circumstances, the investigator wishing to assess and test relatively specific phenotypes should be guided by an explicit model of nicotine dependence. Figure 3.1 depicts a model based on distal measures of nicotine dependence, but if the models are needed to support an assessment of endophenotypic and transitional phenotypes, then causal paths should be articulated. These paths arise out of the available theories and data linking genetic variants with a developmental trajectory comprising (1) the endophenotypes that place a person at risk for experimentation and risk for initiation and progression to nicotine dependence, (2) the transitional phenotypes that become relevant once tobacco use commences, and (3) mechanisms via which endophenotypes and transitional phenotypes are related to clinical/distal measures of nicotine dependence and to mature subphenotypes.

Table 3.3 depicts a working model of how particular genetic variants might manifest different endophenotypes, transitional phenotypes, mature subphenotypes, and ultimately, distal phenotypes. These causal pathways are offered as illustrative exemplars and are not meant to constitute a complete model of nicotine dependence. However, there is some evidence that supports each causal pathway and its phenotypic markers. For example, all of the genetic variants have been linked to either smoking or nicotine dependence, or to constructs strongly implicated in risk for smoking or nicotine dependence, such as for nicotinic receptors/nicotine reward (e.g., *CHRNB2*),[82] for taste sensitivity *(PTC),*[44] for the dopaminergic mechanisms/ incentive salience and sensitization (e.g., *DRD2, DRD4, SLC6A3*),[185–187] and metabolic capacity (e.g., *CYP2D6, CYP2A6*).[188–190] Other relevant constructs might be impulsivity linked to decreased cognitive control (e.g., *MAOA*),[181,191] withdrawal (e.g., $GABA_A$ or α7 nAChRs),[192–195] and affective control/stress recovery *(SLC6A4).*[196–199] For purposes of illustration, a possible genetic influence will be traced across several selected causal paths.

Incentive Salience and Sensitization

Considerable evidence indicates that craving, especially cue-induced craving, appears to be related to activity in dopaminergic systems.[63,200] This is indicated, in part, by responsiveness of dopaminergic, mesotelencephalic structures, such as the nucleus accumbens, to drug stimuli and drug anticipation in addicted populations.[71,201,202] Dopaminergic responsivity might be reflected by both endophenotypic and transitional phenotype markers. The former would be assessed by functional magnetic resonance imaging (fMRI) assessment of activity in mesotelencephalic dopamine structures in response to anticipation of nonpharmacological reward (before any

lifetime nicotine exposure); transitional or mature subphenotypes could be assessed on the basis of activity in the same brain regions, but in response to anticipation of nicotine delivery to smokers.[202] These phenotypic variants, as well as more distal disease phenotypes, could be tested for their association with genetic variants that code for structural and functional properties of the dopamine system (e.g., *DRD2, SLC6A3,* and *DRD4* genes).[185,186] Such variants have been associated with smoking,[203] supporting their potential involvement in at least a subset of habitual smokers.

Cognitive Control and Impulsivity

With respect to the cognitive control/impulsivity causal path, a pattern of violence and impulsivity has been associated with a variable number tandem repeats polymorphism in *MAOA,* which encodes a key enzyme for the catabolism of serotonin and other neurotransmitters. The low-expression variant interacts with stressful experiences that occur early in life, with the combination predicting violent offenses in males.[191] Imaging studies link this low-expression variant with poor integration among the amygdala, the subgenual and caudal portions of the cingulate gyrus, and with the orbitofrontal cortex.[181,204] A great deal of evidence implicates the cingulate and prefrontal regions with an integrated cognitive-control system.[205,206] A mounting body of evidence also implicates cognitive-control functioning, and activity in these brain regions, with drug/nicotine motivational processing.[62,201,207] In addition, there is considerable evidence that impulsivity and related constructs, including externalizing psychopathologies, are related to initiation of smoking and intensity of nicotine dependence.[60] Certain laboratory tasks are sensitive to externalizing personality influences and the tendency to develop specific externalizing disorders, (e.g., P300 in the oddball task)[208] and some tasks are sensitive to extreme dominant response focus.[209] Such tasks require no prior exposure to nicotine and, therefore, might serve as sensitive endophenotypic measures.

Genetic risk for reduced cognitive control might also be associated with transitional patterns such as the tendency to show rapid escalation once smoking begins.[210] Whether the candidate genetic variant and associated externalizing tendencies are causally determinant of initiation per se, or both initiation and nicotine-dependence development, must be elucidated by future longitudinal molecular genetics research. Thus, it is possible that some genetic variants might influence some upstream nodes of the causal chain (initiation) but not later causal processes directly leading to severe nicotine dependence.

Affective Coping

The affective coping causal path might be linked to *SLC6A4,* which contains a variable number tandem repeats variant in the 5′ promoter region *(5-HTTLPR),* with reduced transcription with the short (S) allele relative to the long allele. Research shows that individuals with the S allele tend to report more internalizing symptoms or traits, and this predicts greater likelihood of depression in response to environmental adversity. In addition, evidence suggests that S allele carriers show unusually high levels of amygdala activity in response to stressors consistent with an inability to modulate affective reaction.[197,198] Interestingly, the subgenual cingulate provides inhibitory feedback to the amygdala to regulate processing of environmental threats or stressors. In addition, studies have shown that the S allele carriers show reduced coupling of the subgenual cingulate and the amygdala, which was, in turn, associated with degree of internalizing symptoms.[211] In sum, results suggest that possession of the S allele leads to weakened integration and inhibitory feedback from the subgenual cingulate and the amygdala, and this is

associated with reduced affective control (e.g., fear extinction).

In theory, an inability to control affective response may render a person more dependent on a drug for affective control. Affective control is a primary motive offered by dependent smokers,[50] and this is especially true of smokers with internalizing symptoms. A dependence on smoking for affective control may be one reason that individuals with internalizing disorders smoke more cigarettes than do other smokers and are more likely to be diagnosed with nicotine dependence.[35] In addition, both affect and cognitive-control mechanisms appear to account for urge occurrence,[62] suggesting that smokers with impaired cognitive control, and a resultant inability to cope with negative affect via endogenous control mechanisms, may be especially likely to experience high levels of negative affect and urges and to relapse in response to stressors.

Thus, the available evidence suggests that the relevant effects of the S allele variant of *SLC6A4* may be detected with endophenotypic measures, such as the emotional Stroop paradigm,[212] and fMRI or startle-probe measures of affective responsivity in response to stressors. Moreover, S allele carriers may report higher scores on the WISDM Negative Reinforcement subscale and show higher levels of relapse in response to stressors.

In sum, table 3.3 contains examples of strategies that might be used for genetic mapping before nicotine exposure (endophenotypic markers) and during the periexposure and postexposure periods (transitional and mature subphenotypic markers). The table collapses across transitional and mature subphenotypes for ease of exposition. The table makes clear that all genetic variants and the processes that they encode may lead to elevated levels on the distal phenotype measures (but this is not to say that there will not be significant variation on such measures as a function of differences across the various causal paths). Thus, it is important to note that although two individuals may arrive at the same severity of nicotine dependence according to status on distal measures, the two individuals very likely are not equivalent at earlier stages of the causal chain (including genetic variants). This is why mature subphenotypic measures may detect differences among heavy smokers.

Finally, table 3.3 does not depict how the various causal paths might merge or interact. The table and the associated discussion make clear that causal paths reflect the synthesis of evidence related to functional significance of genetic variants, the constructs and processes related to smoking and nicotine dependence, and knowledge regarding measures that reflect nicotine dependence as well as endophenotype-related processes and other causal mechanisms.

Analytic Strategies

The scope of this chapter is such that approaches to data analysis cannot be discussed in depth. But certain conceptual issues should be mentioned so investigators might bear in mind how such considerations are relevant to their research goals. Data analysis in association studies is escalating dramatically in complexity because of advances in genotyping and the use of more complex phenotypes. For instance, phenotypes are being developed that involve complex developmental-change functions and stage-specific dependence measures. The complex causal models depicted in table 3.3 would also entail complex causal modeling. The discussion of analytic strategies that follows does not address how such complex phenotypes may be most effectively modeled. Chapters 5–9 address issues relevant to such complex phenotypic modeling. However, the discussion below

focuses on how distal phenotypes may be most effectively used in mapping analyses.

Constructing Extreme Groups

As noted earlier, one common strategy for associating genetic variants with phenotypes is via designing extreme groups.[105] In constructing extreme groups, the goal is to achieve a contrast of two groups, one of which comprises a full complement of genetic variants that promote vulnerability for nicotine dependence and has no variants that protect against dependence. The second group comprises variants that protect against nicotine dependence but has no variants that foster dependence. This is, obviously, an ideal that can only be approximated. One assumption in building extreme groups is that genetic vulnerabilities and protective factors cannot be expressed unless individuals have had some level of exposure to nicotine; that is, the assumption is that the genetic variants that affect smoking initiation differ from those that affect the development of dependence.[79,81,82,213]

The review of the nicotine dependence measures and influences presented above suggests one strategy for formulating the two extreme groups. The dependent group members should show high scores on all of the measures listed in table 3.2 for high genetic proneness: they should smoke heavily, pervasively, automatically, and uniformly; smoke early in the day; report severe withdrawal upon attempts to quit; and report an inability to cut down or control their smoking. The nondependent group should, of course, have none of these features.

Both groups should have had some exposure to nicotine, although how extensive that exposure must be is unclear. If the investigator believes that there is a true nicotine-dependence taxon, then the genetic variants particular to that taxon might be best captured by a contrast of two groups with extensive smoking histories—for instance, two groups comprising those who have smoked daily for at least one year. This would ensure that the two groups differ on variants that are relatively specific to dependence per se. To the extent that the groups differ greatly on exposure to tobacco, the more likely it is that differences in genetic variants may be related to factors that promote or retard smoking initiation per se versus dependence. Thus, it is incumbent upon the investigator to decide how specifically to target severe dependence per se in the search for genetic variants.

In forming extreme groups, investigators may wish to consider the timing of nicotine exposure in addition to the sheer amount of exposure. In keeping with the review of gene-environment interactions, investigators may wish to incorporate the notion that all individuals contrasted (in both of the extreme groups) have exposure to tobacco relatively early in life. This would be based upon the notion that early exposure is needed for the expression of genetic influences.[21,34,162,214] It may be that late-onset experimentation is unlikely to lead to severe nicotine dependence even if an individual possesses genetic vulnerabilities.

Obviously, the investigator would wish to conduct additional analyses of results yielded by an extreme groups design. For instance, the investigator would wish to ensure that the effects of the contrast are not specific to gender and that the effects of early smoking are not produced by that variable's relations with syndromes of disinhibition (which would encourage early drug exposure). Such follow-up analyses would address the generality of the effects obtained and the underlying causes of such effects.

The extreme groups strategy is not without its drawbacks. Requiring subjects to be maximally divergent on a host of indicators

of dependence, as indicated in table 3.2, may lead to several problems. For example, even if the measures in table 3.2 agree with one another substantially, selecting on the basis of high scores on all of such measures might entail screening of tremendous numbers of individuals to identify subjects who are uniformly high, or low, across the diverse criteria. Not only might this be impractical and expensive, but it also raises concerns about the representativeness of the samples generated. For example, some forms of psychopathology are associated with very severe nicotine dependence;[21,35,118,215] thus, it is possible that selecting extreme subjects might result in the unintentional selection of those with syndromal or subsyndromal comorbidities. This means that one might inadvertently select for genetic variants associated with conditions or dispositions associated with correlates of extreme nicotine dependence but that are not central to the construct. Of course, all of these concerns are related to where one sets cut-scores on the various measures.

An additional concern with constructing extreme groups is that it severely limits the sort of analytic strategies that one can use to explore the nature of the relations of genetic variants with the phenotypic measures.[216] For instance, as Preacher and colleagues[216] observe, use of the extreme groups design precludes characterization of genetic variant and phenotypic relations across the full range of these variables and may produce model misspecification. In addition, even with the use of taxometric procedures or signal detection methods, it is difficult to know where to place cut-scores to ensure that the dependent group exclusively comprises dependent individuals but does not entail excessive screening. Also, such designs entail the possibility of classification error beyond the usual measurement error assumed with classical test theory. Thus, the procedure may reduce reliability in ways that would be difficult to detect and correct. In particular, the use of extreme scores may yield classification error due to regression to the mean as such scores are likely to be unstable across time. Of course, the use of multiple classification criteria would protect against this threat somewhat. Finally, it is true that extreme groups designs confer greater power and, therefore, are more likely to lead to higher likelihood of statistical significance relative to continuous designs with similar *N*s. However, these designs may produce significant effects even though effect sizes are trivial. Therefore, it seems advisable to use extreme groups designs in exploratory research—when a research area is in its infancy, costs are high, and a premium is placed upon detection of possible effects rather than an estimation of their magnitude. Of course, one should use an extreme groups design if it is clear that a nicotine-dependence taxon really exists,[100] because the nature of the design would match the true distribution of nicotine dependence. However, a good deal of research suggests that differences among smokers may be explained on the basis of different *intensities* of a single dimension, rather than reflecting distinct, qualitatively different types.[102,118–120] (See also Muthén and Asparouhov 2006.[39])

One last observation about the structure or nature of nicotine dependence is relevant here. Questions about the structure of psychiatric disorders—whether it is taxonic or continuous—are very difficult to resolve. For example, it is possible that a continuous dimension may appear categorical or taxonic, while a categorical dimension may appear to be continuous, depending on scaling and measurement properties of the variables involved.[217] This means that even though an item generates scores that suggest the presence of two distinct groups of individuals, such a pattern may be caused by peaked indicators of a continuous dimension. Moreover, it is also the case that the categorical/continuous distinction is a false dichotomy. As Haslam and Kim note, "matters of kind and matters of degree,

itself [might] be a matter of degree."[218(p311)] Thus, nicotine dependence might be caused by a certain all-or-none genetic influence coupled with other graded genetic and environmental influences. In short, distinguishing the underlying structure of a disorder is a complex and difficult undertaking; thus, no definitive conclusions are possible at this time. The bottom line is that whenever investigators use an extreme groups design, they should be aware of the limitations and assumptions entailed.

Alternatives to Extreme Groups Classification

One strategy takes advantage of a theoretically guided selection of criterion measures; it reflects the multifactorial nature of nicotine dependence but does not require that individuals have uniformly extreme scores on all measures. This strategy involves combining measures so they reflect a linear dimension of nicotine dependence. One challenge with a combinatorial strategy is how to achieve proper or appropriate weighting of the predictors. Since the true relation between each nicotine-dependence indicator (criterion) and polymorphisms will likely be unknown, the investigator must devise a system for weighting the various predictors or criteria used to select subjects. Numerous strategies for this are possible.

One strategy is to construct an improper linear model[219] that comprises the principal nicotine-dependence indicators that the researcher believes will tap the major nicotine-dependence facets. In this approach, the researcher can use unit weighting, or weights based on substantive considerations, to create a composite—that is, adding subjects' scores across the set of variables to create a somewhat continuous index of risk. Weights might reflect correlations among the nicotine-dependence criteria, heritability estimates yielded by biometric research, and the importance of the criteria in the investigator's theory of nicotine dependence. This composite would allow for compensatory contributions of variables such that high scores on some elements would compensate for low scores on other measures, and thus, in theory, all subjects could be included in analyses.

Further, the investigator might examine associations of selected polymorphisms with extremes on the composite dimension or on a quasi-continuous dimension constituted of quintiles or deciles of risk. If promising relations ultimately are found in such analyses, these relations could be unpackaged by examining relations of polymorphisms with individual elements of the composite. This strategy has several advantages: (1) inclusion of multiple indices of nicotine dependence; (2) potential to weight the nicotine-dependence indicators (variables) in a manner consistent with their theoretical importance or empirical support; (3) potential retention of most or all subjects; (4) ability to examine relations across a quasi-continuous distribution; (5) ability to unpackage relations with composite elements; and (6) a composite that, no doubt, is more reliable than any single composite element—a desirable feature when dealing with potentially unreliable categorical variables.

The model just presented is compatible with the view that (1) nicotine dependence is fostered or permitted by multiple polymorphisms; (2) persons with nicotine dependence are heterogeneous with respect to which polymorphisms are present (few or none are necessary and sufficient) due, in part, to the fact that some phenotypic measures are more relevant to some nicotine-dependence cases than to others; (3) different nicotine-dependence measures or criteria are modestly intercorrelated, reflecting somewhat distinct causal influences, including genetic influences; and, therefore, (4) the greatest likelihood of capturing a full complement of variants

promoting nicotine dependence is to use a composite comprising multiple nicotine-dependence criteria that index the various relevant polymorphism. If this approach is used, it should capture relevant genes even in the presence of epistasis and pleiotropy or genetic heterogeneity. Such potential causal influences could be examined when the composite measure is unpacked and its elements related to individual genotypes/haplotypes.

The analytic strategies outlined thus far have all been highly theory driven. Of course, an alternative to a theory-based approach is to use empirical search strategies or data-driven approaches[220] to uncover gene, intermediate, and phenotype associations. It is possible that a lack of guiding theory, and a virtually limitless number of potential associations, might yield fortuitous associations and lack of replication.[221,222] Moreover, a strongly empirical strategy merely forestalls the need to hypothesize biological mechanisms and achieve theoretical integration.

Other new approaches to data analysis combine strengths of a priori theoretical and empirical approaches. The approaches have been fostered by challenges and complexities posed by advances in genotyping, as well as increased recognition of the difficulties in modeling complex phenotypes. These complexities become even more pronounced with the "omicization" of observational studies and the availability of new genome-wide technologies for genomics, transcriptomics, metabolomics, proteomics, and so forth.[223] Although much of this chapter has been focused on refining the phenotype and exploring endophenotype definitions, one must also pay close attention to how one measures the biologically relevant factors. Mimicking the move from the evaluation of a single polymorphism to evaluating haplotypes within a single candidate gene, the field is rapidly moving from the evaluation of a single candidate gene to the investigation of numerous gene regions either within a suspected etiologic pathway or even on the genome-wide scale. Combine this wealth of genetic information with potential intermediate measures, biomarkers, environmental factors, endophenotypes, and distal phenotypes, and the "curse of dimensionality," and multiple comparison issues quickly dampen any hope of a statistically significant result with conventional tests of association. However, in a similar fashion to how the watershed model (figure 3.2) provides a structure for the nicotine-dependence phenotype, structure for this statistical analysis can be gained via knowledge of the biology. Although standard multivariate techniques, such as linear regression, are one way of providing structure by limiting the analysis to main effects, two-way interactions, and so forth, these techniques quickly reach their limit with sparse data bias and unstable estimation when the number of terms approaches the number of individuals.[224,225] This difficulty often forces the investigator into choosing a reduced or "best" model via a stepwise selection criterion—a procedure well known to lead the analyst astray of the true model and that, furthermore, does not include the uncertainty in model determination in final inference.[226,227]

As an alternative, one may use hierarchical modeling and Bayesian model averaging to inform final inference via statistical modeling.[228,229] Hierarchical modeling treats the coefficients from a regression model as random effects and incorporates known information about the relations among factors.[230–233] This specifies a joint distribution that both stabilizes the final effect estimates and incorporates dependencies across multiple tests of association. In contrast, Bayes model averaging seeks to use prior knowledge to guide a stochastic model selection approach to models that include more biologically relevant terms.[234,235]

Thus, instead of being faced with an impossible number of interacting terms and possible models, the process is reduced by using biological information to inform and guide the search procedure. In the process of the stochastic search, the data will serve to update the prior probability and disclose the impact of each factor via the posterior probability of the models selected. If the knowledge specifying the structure of the relations is very well defined, one may use structural equation modeling or, in the case of metabolic pathways, pharmacokinetic models to specify the topology between factors.[236] Of course, such approaches are extremely model dependent and may have serious identifiability problems for intermediate latent variables if the study is limited to genes and distal phenotype measures only. However, one may enhance estimation within this framework by gathering intermediate or endophenotype measures on a small subsample of individuals and then performing a combined analysis (main study and substudy) to inform the latent structure of the topology.

Finally, in an extreme example of using the known biology and sampling schemes to inform an analysis, a novel approach is to adopt a Mendelian randomization approach in which genetic variants are used to make inferences about critical intermediates.[183,237,238] The basic idea is that if a causal pathway is correctly specified, then the effect of an intermediate factor on an outcome can be estimated through the ratio of coefficients of the regression of the outcome on the gene and of the intermediate on the gene. Thus, the gene acts as a randomized control for the intermediate's effect on the outcome, helping to protect against reverse causation and unknown confounding effects.

An example of this might be if one or more genetic variants were found that resulted in high levels of cotinine to accrue in response to nicotine use. If this were found to relate strongly to the nicotine-dependence phenotype, one might infer that a high level of cotinine is an important pathogen (at least for some people, if it is a moderator). Thus, in a gene-environment interaction approach, this would suggest a gene-gene interaction that would allow one to make inferences about a gene-environment interaction. Although the use of biology guiding statistical analysis is not new, the direct incorporation of that knowledge into statistical inference is still in its infancy. Questions still remain as to what kind of information is most relevant and how much of the final inference is dependent on prior structuring of the relations and topology.

Summary

One's model of nicotine dependence guides decisions about the measurement of the nicotine-dependence phenotype—whether such guidance is explicit or implicit. It is best if the investigator makes such guidance explicit by clearly articulating hypotheses about the nature of dependence processes, the biological origins of such processes, and how they manifest phenotypically. Research suggests that while nicotine dependence is multidimensional, measures that assess heavy, pervasive, and automatic smoking appear to capture core variance related to this construct. However, the investigator should consider how to incorporate the assessment of complementary dimensions of dependence, how to control for error in dependence assessments, and whether to incorporate gene-environment interactions into attempts at genetic mapping. More thorough assessment of the dependence construct may require the development of new dependence assays that are focused on relatively discrete biological mechanisms. In addition, a comprehensive portrayal of nicotine dependence may require the development of measures of intermediate and transitional phenotypes that capture processes in dependence across its

development. The investigator must also decide on how to integrate a model and assessment of dependence with particular experimental and analytic strategies. This endeavor should also be guided by the investigator's explicit hypotheses about the nature of dependence and how it should manifest across persons and across the developmental process. Finally, it is important to note that numerous theories of nicotine dependence are possible, and the investigator should systematically examine competing and distinct models.

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.

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