An Integrative Approach for Studying the Etiology of Alcoholism and Other Addictions

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Studies of alcoholism etiology often focus on genetic or psychosocial approaches, but not both. Greater understanding of the etiology of alcohol, tobacco and other addictions will come from integration of these research traditions. A research approach is outlined to test three models for the etiology of addictions — behavioral undercontrol, pharmacologic vulnerability, negative affect regulation — addressing key questions including (i) mediators of genetic effects, (ii) genotype-environment correlation effects, (iii) genotype x environment interaction effects, (iv) the developmental unfolding of genetic and environmental effects , (v) subtyping including identification of distinct trajectories of substance involvement, (vi) identification of individual genes that contribute to risk, and (vii) the consequences of excessive use. By using coordinated research designs, including prospective assessment of adolescent twins and their siblings and parents; of adult substance dependent and control twins and their MZ and DZ cotwins, the spouses of these pairs, and their adolescent offspring; and of regular families; by selecting for gene-mapping approaches sibships screened for extreme concordance or discordance on quantitative indices of substance use; and by using experimental (drug challenge) as well as survey approaches, a number of key questions concerning addiction etiology can be addressed. We discuss complementary strengths and weaknesses of different sampling strategies, as well as methods to implement such an integrated approach illustrated for the study of alcoholism etiology. A coordinated program of twin and family studies will allow a comprehensive dissection of the interplay of genetic and environmental risk-factors in the etiology of alcoholism and other addictions.

For much of the past two decades, family studies of alcoholism have been conducted by two relatively nonoverlapping research groups: psychosocial researchers with interests in family environmental variables (e.g., parent-child relationships, family rituals) that interact with socio-environmental variables (e.g., peer relations) as well as individual difference variables (e.g., impulsive, inattentive or antisocial behaviors: behavioral undercontrol) in accounting for the link between family history of alcoholism and offspring outcome; and behavioral geneticists with interests in estimating genetic contributions to alcoholism risk and in differentiating the remaining variance into shared and nonshared components (Heath, Slutske et al., 1997; Jacob & Leonard, 1994). Psychosocial and genetic studies of tobacco and illicit drug dependence have been even more poorly integrated (Heath & Madden, 1995). From the psychosocial research tradition, there has developed a rich literature of theory and findings which has offered increasingly sophisticated models of etiology, defined a number of key mediator and moderator mechanisms that may account for or qualify the impact of family history risk on offspring outcome, and produced a number of high-quality, longitudinal data sets aimed at testing alternative models of mediation and moderation. The major shortcoming of this line of research is one of indeterminancy of findings, since all such efforts have involved passive longitudinal designs (i.e., family studies) and hence separation of family genes from family environments has not been possible. In contrast, behavioral genetic studies of the past twenty years have offered an increasingly persuasive argument that genetic influences ultimately account for 40-60% of the variance in alcoholism risk, and that the remaining variance is only partly explainable in terms of shared family environmental effects (Heath, 1995b; Heath, Slutske et al., 1997). Similar conclusions have been reached regarding the importance of genetic influences for the etiology of smoking and tobacco dependence (Heath & Madden, 1995; Heath et al., 1998; Kendler et al., 1999) and illicit drug use and dependence (Kendler, Karkowski et al., 2000; Kendler & Prescott, 1998; Tsuang et al., 1996).

The strength of the above conclusions, however, must be qualified in light of several notable limitations associated with this literature. First, family and other shared environmental (as well as nonshared environmental) influences have been poorly articulated and measured by behavioral geneticists; such environmental effects can be detected with much greater power if individual risk-factors are explicitly assessed and included in analyses. Second, very little is known about how genetic effects are mediated and moderated by environmental influences – that is, the nature of gene-environment correlations and gene-environment interactions relevant to addiction etiology and course. Failure to assess pertinent shared environmental risk-factors may be especially problematic for studies of moderator influences (i.e. genotype x shared environment interaction effects), since in the classical twin design these interaction effects, unless explicitly modeled, will be confounded with the genetic main effect (Eaves et al., 1977). Third, the qualifying effects of different alcoholism (or other psychoactive substance dependent) subtypes in terms of heritability estimates are not yet clearly understood (Heath, Slutske et al., 1997) and the nature of direct and indirect spouse influences (assortative mating or spousal interaction) in contributing to offspring risk remains to be determined (Heath, 1987; Heath & Eaves, 1985). Finally, the impact of childhood events and behaviors *and* of young adult events and behaviors in qualifying and/or explaining risk have not been adequately explored within a genetically informative research design.

To move beyond the current knowledge in this area, our research group has attempted to emphasize and integrate prospective/developmental, high-risk, behavioral genetic, genetic epidemiologic and experimental perspectives in our studies of alcoholism etiology leading to the development of a multi-site collaborative program of research. The purpose of this paper is to review the major features of this approach — its rationale, conceptual foundation, and primary sampling strategies — in an effort to communicate to the addiction and behavioral genetic research communities the great potential that is embodied in such an integrated approach. In so doing, we hope to provide a paradigm for the ways in which family and twin-family studies can provide critical insights into the etiology of complex disorders such as alcoholism as well as tobacco and illicit drug dependence.

Method

Overview

The multi-site Missouri Alcoholism Research Center (MARC) grew out of the inter-linked research collaborations between researchers at Washington University School of Medicine in St. Louis, Saint-Louis University School of Public Health, the University of Missouri at Columbia, the Palo Alto VA Medical Center, and the Queensland Institute of Medical Research, Brisbane, Australia. As well, international collaborations with researchers in Scandinavia are being established to address cross-cultural or other research questions that cannot easily be addressed in the U.S. The broad focus of our research concerns the etiology and course of alcoholism, tobacco dependence and other associ-

ated drug problems, and psychiatric comorbidity. During the Center's early years, most projects have focused on samples of adolescents and young adults, the age when substance use disorders most commonly develop (Nelson et al., 1998). Center-wide research themes involve testing three interrelated classes of mediational model for genetic and environmental influences on addiction risk — (a) behavioral undercontrol; (b) pharmacologic vulnerability; (c) negative affect regulation — with a unique combination of psychosocial, genetic, epidemiologic and experimental approaches. The integrating theme of the MARC is that such mediational models can best be tested in prospective family studies, involving genetically informative research designs, and a multi-disciplinary approach.

The conceptualization of the Center, and design of individual research projects, was guided by the conviction that we need to begin the process of integrating psychosocial, behavioral genetic, molecular genetic, high-risk and epidemiologic research on alcoholism and associated substance use disorders; and that the greatest progress can be made through a series of coordinated prospective, high-risk family and behavioral genetic studies that include collection of DNA samples for future genotyping, and experimental studies that incorporate these multiple perspectives. With such an integrated approach, it should be possible to achieve an increasing convergence of disparate research traditions (a goal that will become increasingly important as the field identifies more individual genetic loci that contribute to risk of alcohol dependence through projects such as the multi-site Collaborative Study of the Genetics of Alcoholism (COGA; Reich et al., 1998), and thereby generate important advances in our understanding of the relationships between risk of alcohol, tobacco and other psychoactive substance use disorders and (a) behavioral undercontrol, (b) depression and anxiety disorders, and (c) individual difference factors leading to differences in pharmacologic vulnerability.

Major Research Themes: Mediators of Alcoholism Risk and Risk-Modifiers

The scientific rationale underlying our major research themes is focused on three classes of mediational models (Baron and Kenny, 1986) and their associated risk-modifiers and interactions. By a *mediator* we mean a variable that accounts for part of the relationship between a predictor variable (observed or latent) and an outcome variable, so that when the mediating variable is controlled for, the association between the predictor variable and the outcome variable is reduced. Much psychosocial research on alcoholism attempts to identify mediators (intervening variables) that account for the relationship between family history of alcoholism and offspring alcoholism or other outcomes. The past decade has seen several high quality studies conducted that address the issue of potential mediators (Chassin et al., 1993; Newcomb & Felix-Ortiz, 1992; Sher et al., 1996; Windle, 1990). A behavioral genetic approach seeks to extend this one step further, by separately identifying the mediators of genetic effects, as well as of familial environmental effects, and non-shared environmental effects on alcoholism risk (Heath et al., 1993). Such

an approach recognizes that the proximal mediators of genetic effects on risk may in fact be environmental (e.g., peer influences) — an example of genotype-environment correlation. Despite the extensive literature on the genetic contribution to alcoholism risk (Heath, 1995b; Heath, Slutske et al., 1997), studies of mediators of genetic effects are only in their infancy.

By a *risk-modifier* we mean a variable that interacts with a mediating or predictor variable to affect outcome risk, such that the relationship between the mediating/predictor variable and the outcome variable is much stronger at some (e.g. high) values of the risk-modifier than at others. Psychosocial researchers have often used the term "moderating variable" or "vulnerability factor" to describe risk-modifiers, but we prefer the more neutral term which carries no linguistic implication of direction of effect. For geneticists, the notion of a risk-modifier has long been familiar under the guise of genotype x environment interaction - the effect of an environmental risk factor may vary as a function of genotype, or conversely the effect of a genetic risk-factor may vary as a function of environmental variables. For example, because of increased social pressures to drink after work, Japanese heterozygotes with one ALDH2*2 null allele, who experience a flushing response to alcohol, appear to be at disproportionately greater risk of alcohol dependence in contemporary Japan than they were 20–30 years ago (Higuchi et al., 1994). The same concept also applies to environmental (ie. non-genetic) modifiers of environmental effects on alcoholism risk.

Major Alcoholism Etiology Models

We will focus our discussion on three major models of alcoholism etiology. While neither comprehensive nor mutually exclusive, these three models provide a useful framework for highlighting issues in studying the etiology of a complex disorder such as alcoholism, and lead to insights into the ways in which an integrated program of twin-family studies can provide critical data for testing etiologic hypotheses. While we focus our attention on the etiology of alcoholism, the same models contain many elements with application to tobacco and other drug dependence.

Model 1: Behavioral Undercontrol. Aspects of behavioral under-control (e.g. history of conduct problems, or adult antisocial behavior) are some of the most potent predictors of alcohol problems in both women and men (Kessler et al., 1997; Regier et al., 1990; Sher, 1991), and arguably the strongest mediators of genetic effects on alcoholism risk in both genders (Heath, Bucholz et al., 1997; Slutske et al., 1998). In the context of psychosocial research, this model is often referred to as the "deviant socialization" model. Briefly, a "difficult" temperament (Blackson et al., 1996; Thomas & Chess, 1977), in combination with ineffective parental control associated with parental alcoholism (Dishion et al., 1998), is hypothesized to lead to undersocialized behavior resulting in poor academic adjustment and school failure. Associated cognitive or behavioral deficits are also posited to contribute to school failure, which in turn leads to association with deviant, substance abusing peers, increased conduct problems, and substance involvement. Direct temperament effects on peer selection are also posited, since impulsive individuals may be more likely to seek out peers with similar traits. Thus the deviant socialization model implicates (a) social learning factors such as impaired parenting (Chassin et al., 1993; Dishion et al., 1998), family disruption (Steinglass et al., 1987), and peer influence (Chassin et al.1993), and (b) individual difference factors such as temperament/personality (Blackson et al., 1996), and externalizing disorders such as history of hyperactivity, attentional or oppositional problems (Peterson & Pihl, 1990; Pihl & Bruce, 1995). Both classes of variables are related to a family history of alcoholism and a range of problem behaviors including alcohol misuse (Pihl et al., 1990; Sher, 1991; Windle & Searles, 1990). This social learning theory approach (implicit in the "deviant socialization" model) can be tested with considerable power using a behavioral genetic paradigm. It is our assessment that it has not yet been adequately tested. An interaction of genetic differences with shared environmental effects such as parenting effects will be confounded with a main effect of genotype in twin pairs reared together (Eaves et al., 1977), unless these environmental variables are explicitly included as measured variables and the interaction is appropriately modeled. Typically, behavioral geneticists have not attempted to model such effects.

Model 2: Pharmacologic Vulnerability. The pharmacologic vulnerability model posits that there are important individual differences in subjective, psychomotor or physiological response to alcohol, which lead ultimately to differences in alcohol dependence risk. Parallel models have been advocated in the tobacco field (Pomerleau et al., 1993). The reduced sensitivity to the effects of alcohol that is observed in Adult Children of Alcoholics (ACOAs) compared to controls (Pollock, 1992) is at least partly under genetic control (Heath and Martin, 1991a,b), is associated with differences in alcohol consumption levels (Neale & Cardon, 1992), and is predictive, at long-term follow-up, of increased risk of alcohol dependence (Heath, Madden, Bucholz et al., 1999; Schuckit & Smith, 1996). More strongly aversive reactions to the effects of alcohol, in the case of Japanese ALDH2*2 homozygotes and heterozygotes, are associated with reduced risk (Higuchi et al., 1994). Levels of alcohol consumption may in some cases be an important mediating variable — ALDH2*2 homozygotes and heterozygotes have substantially reduced levels of alcohol consumption compared to ALDH2*1 homozygotes (Higuchi et al., 1996; Muramatsu et al., 1995) — but other mechanisms may be involved. For example, in individuals of Asian ancestry, the ADH2*2 allele at the alcohol dehydrogenase ADH2 locus is also associated with differences in alcohol dependence risk, but has not been found to have a significant effect on consumption levels within the normal range in Asian control samples (Higuchi et al., 1996; Muramatsu et al., 1995). (However, data on Australian males of European ancestry do support an association between the ADH2*2 allele and reductions in both alcohol dependence risk and alcohol consumption level in this heavier-drinking society [Whitfield et al., 1998]). Findings from several large-scale twin studies of individuals of predominantly European ancestry confirm a substantial genetic contribution, in both women and men, to differ-

ences in alcohol consumption levels in general population (i.e. predominantly non-alcoholic) samples (Heath, 1995a), and these genetic effects are associated with differences in alcoholism risk, even when personality variables and history of psychopathology are controlled for (Heath, Slutske et al., 1994). Individual differences in alcohol sensitivity may also be translated into increased expectancies of positive reinforcement from alcohol in individuals with sufficient drinking experience; and these expectancies may in turn function as proximal mediators of drinking behavior. The pharmacologic vulnerability model encompasses environmental as well as genetic mechanisms for the transmission of risk. Individual differences in alcohol effects may be mediated in part by individual differences in beliefs about the personal effects of alcohol (Fillmore & Vogel-Sprott, 1995; Nagoshi et al., 1992; Sher et al., 1996) which may be formed out of both direct pharmacologic experience and social learning from sources in the near environment (e.g. family, friends) and the larger culture (e.g. mass media).

Our work on the pharmacologic vulnerability model focuses primarily on four key issues: (a) the extent to which individual differences in self-reported tolerance to alcohol (Schuckit et al., 1997) early in the drinking career predict differences in alcoholism risk, and constitute an important mediator of genetic influences; (b) the extent to which such subjective tolerance measures, in conjunction with measures of personality and history of psychopathology, predict future differences in alcohol expectancies and differences in alcohol consumption patterns; (c) the extent to which cross-tolerance to alcohol in smokers can be demonstrated and (d) whether such cross-tolerance effects predict increased probability of making the transition to heavy drinking, and thus constitute one mechanism by which the strong association between smoking and both heavy drinking and alcohol dependence risk arises. This research focus is motivated by several findings: (i) the work of Collins et al.(1996) who have demonstrated in certain mice strains tolerance to alcohol after acute or chronic nicotine administration; (ii) reanalyses of alcohol challenge data (Madden et al., 1995, 1997) which demonstrated that smoking history predicted lower ratings of intoxication after a challenge dose of alcohol, even after differences in drinking history were controlled for — in other words, that smokers' responses to alcohol were more similar to Schuckit's insensitive Sons of Alcoholics (SOAs) who had been found to be at increased risk of alcohol dependence (Schuckit & Smith, 1996); (iii) other data from the Australian twin study showing increased rates of smoking in non-alcoholics at increased genetic risk of alcohol dependence (i.e., having alcoholic MZ cotwins) (Madden et al., in press).

Model 3: Negative Affect Regulation. In our discussion of affect regulation, we emphasize regulation of *negative* affect, while acknowledging that drinking to enhance positive affect is an important and understudied component of affect regulation (Cooper, 1994; Cooper et al., 1992, 1995). The negative affect regulation model is most commonly used to explain the relationship between negative affect and the development of alcohol problems. It posits that alcohol misuse is motivated by attempts at self-medicating an affective disturbance (depression or anxiety

disorders), and thus, may be associated with high levels of life stress. This model has generated extensive literatures on alcohol-stress interactions (Cappell & Greeley, 1987; Pohorecky, 1991; Sher, 1987) and on comorbidity between alcoholism and psychiatric conditions such as anxiety and mood disorders (Kessler et al., 1997; Lehman & Dixon, 1995; Regier et al., 1990). Many individuals report using alcohol to relieve dysphoric symptoms and endorsement of such reasons is strongly associated with alcohol involvement (Sher, 1987). The literature on alcoholism and personality also suggests associations between family history, predisposition to experience negative emotional states, and alcohol problems; however, this literature is ambiguous (Sher & Trull, 1994). Studies indicating that a range of childhood stressors (e.g., sexual and physical abuse) are associated with having an alcoholic parent (Famularo et al., 1992) and with later alcohol problems (Dinwiddie et al., 2000; Kendler, Bulik et al., 2000; Miller & Martin, 1993) are at least consistent with an affect regulation model, although other hypotheses cannot yet be ruled out. Interpretation of these literatures is complicated by a number of methodological factors including problems in sampling and ascertainment, frequent use of retrospective reports, failure in clinical samples to distinguish clearly between depression and the affective consequences of alcohol withdrawal (Schuckit, 1994), and the inability to rule out confounds such as gene environment covariation (Widom et al., 1993). Finally, there appear to be large individual differences in drinking in response to stressors and aversive emotional states (Kushner et al., 1994) which, if ignored, could lead to underestimation of the role of affect regulation as a mediator of alcoholism risk.

Towards an Integrated Model

In an attempt to integrate hypotheses about the interrelationships between the behavioral undercontrol, affect regulation and pharmacologic vulnerability models, we propose a strong heuristic model which distinguishes between two factors, (i) level of alcohol exposure, and (ii) the threshold above which an individual is at a high risk of becoming dependent. It is hypothesized that the former is strongly determined by aspects of behavioral undercontrol and associated environmental risk-factors (e.g., peer substance use), and the threshold for the development of alcohol problems by history of depression or other measures of behavioral inhibition. It is hypothesized that some genetic factors will contribute to differences in alcohol dependence risk via effects on level of alcohol exposure, while other genetic factors will influence alcohol dependence vulnerability, i.e. the threshold of alcohol exposure above which an individual is at high risk of becoming dependent. Thus individuals with identical histories of alcohol consumption are hypothesized to differ in their alcohol dependence vulnerability (with those with a history of depression at increased risk) while individuals with the same "threshold" of vulnerability may differ markedly in risk because of differences in drinking history. Differences in alcohol reactivity, as assessed using standard alcohol challenge paradigms, and in effects of smoking, are hypothesized to lead to differences in alcohol exposure,

while other differences in pharmacologic response may affect vulnerability, or may have effects on both exposure and vulnerability. Thus the ALDH2*2 allele in individuals of Asian ancestry is associated with both decreased exposure, i.e. reduced average levels of exposure (Higuchi et al., 1996; Muto et al., 2000) but also apparently with increased vulnerability, in the form of increased risk of liver disease and alcohol-related cancers in those who progress to become heavy drinkers (Couzigou et al., 1994; Yokoyama et al, 1996, 1998)

Key Etiologic Questions and Alternative Research Designs

In this section, we consider some of the major etiologic hypotheses that need to be addressed in order to understand the familial transmission of alcoholism. (For a more detailed review, see Heath et al., 1996). Almost all apply equally well to questions about tobacco and other substance dependence (Heath et al., 1998). We then consider the extent to which traditional sampling designs that have been used in psychosocial and epidemiological studies on addiction are adequate for meeting these goals. Finally, we consider the ways in which family and twin-family approaches may be used to resolve some of these research questions.

Major Etiologic Questions. In a previous review (Heath et al., 1996), we identified a number of interrelated questions about the etiology of alcoholism, which are potentially applicable to all three etiologic models reviewed above. These interelated questions and models are applicable to the etiology of tobacco and illicit drug dependence as well.

1. How do genes act to increase alcoholism risk? In effect this is a question about mediators of genetic influence on risk. A parallel question may also be asked about environmental risk-factors.

2. Are individuals at high genetic risk also more likely to be exposed to high risk environments? In effect, this is a question about genotype-environment correlation (Eaves et al., 1977). As reviewed by Eaves et al., genotype-environment correlation can arise through many different mechanisms, having consequently differing implications for risk of alcoholism or other substance use disorders. There may be G-E correlation because individuals at high genetic risk may select out high risk environments; for example, individuals who are high on impulsivity or related traits (which are at least partly under genetic control; Martin et al., 1979) may be more likely to develop problems with alcohol. Alternatively, these may be G-E correlation because family members, who are genetically related, also have environmental influences on an individual's risk. Thus, alcoholic parents may transmit increased genetic risk of alcoholism to their offspring and also expose their offspring to higher-risk environments than non-alcoholic parents (e.g., greater likelihood of experiencing early childhood trauma; or greater likelihood of exposure to maternal pregnancy substance use). Finally, G-E correlation may arise because of assortative mating or assortative friendship (if alcoholics are more likely to mate with other alcoholics; Hall et al., 1983a,b) or because other heritable traits are associated with alco-

holism risk, and are also susceptible to environmental influence by that partner or friend. This second question essentially concerns the interrelationship between mediators of environmental and of genetic effects on risk.

3. Under what conditions is the impact of high genetic risk exacerbated or reduced by vulnerability or protective factors (genotype x environment interaction effects; Eaves et al., 1977). These conditions may include interactions with cohort or gender as well as with environmental risk-factors (e.g., parenting behaviors or school, neighborhood and other sociocultural effects). All of these issues relate to risk-modifier effects, whereby the genetic contribution to risk may be much greater under some environmental conditions than under others.

4. How do genetic and environmental influences unfold through the course of the individual's development (Eaves et al., 1986) to determine the natural history of drinking and alcohol-related problems? More specifically: (i) are there stages in the onset and course of alcoholism, with stage-specific genetic or environmental risk factors; (ii) to what degree do genetic versus environmental factors determine persistence versus remission of alcohol problems over time?

5. Can we identify alcoholic subtypes, or distinct trajectories of alcohol use, with distinct modes of inheritance or type-specific risk-factors (Bucholz et al., 1996; Heath, Bucholz et al., 1994)?

6. Can we identify individual genes that contribute to alcoholism risk, and characterize their mode of action and interaction with environmental risk-factors?

To this list we may add an additional question:

7. What are the consequences (in terms of health, or other aspects of physiological functioning) of alcoholism or excessive alcohol use or other substance use, and how are these effects moderated by individual genotype?

Within each of our three major etiological models, substance dependence and chronic and excessive use may in turn influence behavioral undercontrol, negative affect, and subjective or physiological responses to alcohol, tobacco or other drugs. Causal influence cannot be assumed to be unidirectional.

As in the case of the three major classes of etiologic model, these seven research questions are neither comprehensive nor mutually exclusive. Questions about genotype x environment interaction and genotype-environment correlation, for example, are posed separately but these processes almost certainly often occur concurrently. These seven issues, however, do outline a matrix of etiologic questions (three classes of etiologic model x seven research questions) that help focus our efforts to understand the etiology of alcoholism. The dimensionality of this matrix may be further increased by recognizing that the same processes cannot always be assumed to be operative for different developmental stages, genders, or ethnic groups. To date, too much of our thinking about alcoholism etiology has

unfortunately been based on studies of adult or college-age males of European ancestry.

Limitations of Traditional "High-risk" Design

The conventional high-risk research paradigm has made (and will continue to make) important contributions to our understanding of the etiology of alcoholism. Foremost among such approaches has been the comparison of individuals stratified by family history of alcoholism (for a review, see Sher, 1991), and studied either cross-sectionally or longitudinally. Individuals with and without a family history of alcoholism are compared.

Several limitations characterize this type of design which must be considered when interpreting results. Typically, family history is assessed only by respondent report, which may introduce systematic biases (for example, those with a history of heavy drinking may be more likely to know about or recognize alcohol problems in their relatives). Furthermore, such assessments are usually limited to family history of alcoholism, although other psychiatric disorders that are comorbid with alcoholism (i.e. co-occur with it at a higher than chance rate) may in fact prove to be responsible for differences between high-risk and control groups. Important mediating variables may be identified which prove to be mediators for the wrong disorder! Frequently, those with a maternal history of alcoholism are excluded because of possible effects of maternal drinking or other substance use during pregnancy on outcome measures. Yet having an alcoholic partner may be associated with greater likelihood of drinking, smoking or other high-risk behaviors during pregnancy, even in families where the mother does not meet criteria for alcohol dependence, a confounding factor that is unlikely to be discovered without direct assessment of the mother. The absence of detailed information regarding spouse characteristics also means that conclusions about genotype-environment correlation effects due to assortative mating must be based upon family history reports, and therefore are of uncertain validity. And more generally, parental assessments make inferences about genotype x environment interaction effects hazardous - since these effects will typically be inferred on the basis of stronger parent-offspring resemblance under high-risk than low-risk environmental exposures, a difference that may alternatively be accounted for by simple reporting bias (for example, if alcoholic women are more likely to acknowledge maternal alcohol problems if they also experienced abuse or neglect as a child).

In addition, most often such studies have assessed only a single offspring per family, a design feature which is associated with important limitations. First, the detection of genetic dominance effects (which contribute to sibling but not to parent-offspring resemblance; Bulmer, 1980) will not be possible, so that the overall genetic contribution to alcoholism risk may be underestimated. For the analysis of genotype x environment interaction, having direct assessments on two or more siblings per family, with stratification of families by level of environmental risk, provides a powerful test for such effects: stronger genetic influence, and hence higher sibling resemblance, would typically be predicted under high-risk environmental conditions. Perhaps most importantly, this literature has often erroneously used temporal sequencing of variables associated with offspring alcoholism to infer causality (Kandel et al., 1992), for example, suggesting a causal link between conduct disorder and alcohol dependence based on the observations that offspring of alcoholics vs. non-alcoholics are more likely to exhibit conduct disorders, that conduct disorder is more common in those who become alcohol dependent, and that conduct disorders typically occur earlier than the development of alcohol dependence symptoms. In the conventional high-risk paradigm, we have no way of testing the strong alternative hypothesis that the two disorders share common unmeasured risk-factors with no direct causal influence of one disorder on the other. With assessment of multiple siblings in a family, we can at least ask the question of whether in sib pairs discordant for conduct disorder, it is the sibling with a history of conduct disorder who is more likely to develop problems with alcohol or other drugs. If the co-occurrence of alcohol and conduct problems is solely due to shared environmental risk-factors common to both disorders, no differences in risk of alcohol problems would be predicted in these discordant pairs. If the two disorders shared genetic as well as environmental risk-factors in common, then on average the sibling with a history of conduct disorder (who on average will have higher genetic risk) will still be more likely to develop alcohol problems than the sibling without, whereas in MZ twin pairs discordant for conduct disorder (who are of course genetically identical) there should be no differences in alcoholism risk. Thus for inferences about mediating variables (including genotype-environment correlation effects) and for inferences about influences on developmental course, assessment of additional siblings in a family provides an important check of strong assumptions that would otherwise remain untested. Questions about resiliency factors, and about consequences of alcohol misuse, can likewise be addressed with much greater conviction when within-family (sibling) controls are available to remove potential confounds due to family background (including genetic) risk-factors.

Finally, even in the identification of individual genes that contribute to addiction risk, the conventional case-control study employed in high-risk research runs the danger of false-positive findings when differences in allele frequency due to differences in ancestral origin are correlated with sociocultural differences. While the importance of such "population stratification" or confounding effects has often been questioned (Morton & Collins, 1998; Risch, 2000), they are likely to be especially plausible for alcohol, tobacco and drug use behaviors which are especially prone to sociocultural influences. For an entertaining example, see Heath, Bucholz et al. (2001) for a discussion of how the ADH2*2 allele, although clearly a functional polymorphism associated with effects on ethanol metabolism, is associated with belief in Socialism in an Australian sample, because both are more common in those of English than those of other ancestries. Once again, one solution to this problem is to be found in the use of within-family controls (Schaid & Rowland, 1998; Spielman & Ewens, 1999; Spielman et al., 1998), although the use of multiple

unlinked markers in an attempt to define population genetic structure and control for such confounding effects is also a possibility (Pritchard & Rosenberg, 1999), albeit one that has not yet been demonstrated in practice to perform adequately in a population as heterogeneous, in terms of ancestral origins, as the U.S., Australia, or most other western societies.

Limitations of Adoption Studies

Some of the most convincing early evidence for genetic effects on alcoholism were provided by adoption study data (Goodwin et al., 1973, 1977), which compared risk of alcoholism in the adopted-away biological offspring of alcoholic versus control parents. Subsequent adoption studies have for the most part supported the importance of genetic influences (Cadoret, 1994; Cloninger et al., 1981). However, for a comprehensive dissection of the role of genetic and environmental factors in the etiology of alcoholism, the adoption study has certain important limitations. Two issues in particular need to be considered: (i) interpreting the absence of shared environmental influences in adoption data is complicated by the fact that adoptive parents are typically screened for the absence of psychopathology, are typically older at the time that they adopt, and are less likely to be experiencing alcohol problems at the time they are rearing children; and (ii) it is rare that the full range of psychopathology in the biologic parents has been well characterized. The latter issue applies even more strongly to research on tobacco use and illicit drug use, information about which is likely to be often missing from adoption records. Thus in adoption data we typically are confronted with poor characterization of genetic risk, and, while environmental history can be assessed in great detail, the range of environmental exposures is likely to be severely limited in adoptees compared to biological children reared by their own, in some cases actively alcoholic, parents. Both these considerations seriously limit the utility of the adoption design for dissecting the interplay of genetic and environmental risk-mechanisms in the case of addictive disorders, and in particular limit our ability to make generalizable statements about the relative importance of different etiologic mechanisms.

The Classical Twin Study and its Twin-sibship Extensions

Much of what can be said about the utility of the twin study has previously been stated by other investigators, notably in elegant early papers by Jinks and Fulker (1970) and Eaves (1982). Because the classical twin study depends upon comparison of pairs of individuals who are matched in age and family background, but either correlated 1.0 (MZ pairs) or 0.5 (DZ pairs) with respect to additive genetic effects, it provides considerable power for detecting genetic effects, including (i) identification of mediators of genetic influence, and resolution of the multivariate structure of genetic effects on multiple outcome measures, (ii) estimation of genotype x environment interaction effects, (iii) testing of hypotheses about the developmental unfolding of genetic effects, and, as noted above,(iv) testing of hypotheses about the consequences of alcohol or other drug use, using discordant pair approaches and their extensions. The classical twin study also provides considerable power

for addressing questions about subtyping, either cross-sectionally by symptom profile, or prospectively by trajectories of substance use or problems (Bucholz et al., 1996, 2000; Cloninger et al., 1981; Eaves et al., 1993; Heath, Bucholz et al., 1994). There is considerable utility for such questions in having pairs of individuals perfectly matched for genotype and family background when attempting to define diagnostic boundaries or refine classification schemes. The twin study also has the advantage of much greater generalizability of findings to the general population than in the case of adoptees, since the full spectrum of genetic and environmental risk will be represented in twins and their families: monozygotic twinning occurs essentially at random, and dizygotic twinning shows only a weak association with older maternal age and lower socioeconomic status (Bulmer, 1970). Finally, in studies where no attempt is made to assess individual environmental risk-factors, and given the limitations of adoption data, the classical twin design remains the most powerful design for detecting shared environmental effects, controlling for genetic effects (Martin et al., 1978).

It has long been recognized that additional advantages are gained by extending the classical twin design by adding siblings of twins (Posthuma & Boomsma, 2000). Where the investigation is attempting to study additive genetic and shared environmental effects on adult phenotypes not previously well characterized with respect to heritability, or mediating variables and genotype-environment correlation effects, or developmental effects that are being addressed in prospective research (such that sibling assessments will eventually be available at the same ages as for the twins), the most efficient design is achieved by adding full siblings of MZ pairs only to the traditional twin design. This provides a check on the generalizability of twin data (by comparing twin-sib and DZ twin pair correlations) and, because there are two twin-sib comparisons for each trio of an MZ pair plus one full sibling, yields a considerable increase in power, per individual assessed, compared to sampling MZ and DZ twin pairs only. Sampling of siblings of twin pairs has also become of considerable potential importance for gene-mapping studies using Quantitative Trait Locus linkage and association mapping approaches focused on quantitative measures (e.g. quantitative indices of alcohol use or problems), and for this purpose siblings of DZ pairs are especially useful. As recently reviewed (Sham et al., 2000), the informativeness of sampling sibships of variable size under random sampling is proportional to $N(N-1)/2$ where N is the number of full siblings in the sibship. Since the DZ twinning rate is increased in older mothers (Bulmer, 1970), sibships of DZ twins are on average larger than most randomly sampled sibships (see for example Table 1 for sibship sizes of families with MZ and DZ pairs from a young adult Australian twin sample (Heath, Howells et al., 2001). Even further gains in power can be achieved when the power of twin-sibship designs is combined with definition of multivariate phenotypes (Martin et al., 1997) or, in the case of small sibship size, when extreme discordant or extreme discordant and extreme concordant sib pairs can be identified from previ-

ously screened populations (Eaves & Meyer, 1994; Gu et al., 1996; Risch & Zhang, 1995, 1996).

The classical twin design however is not without important limitations (Eaves et al., 1978). Without assessment of the parental generation, no information is available about assortative mating in the parents. Some, but not all, mechanisms of assortative mating will increase the genetic correlation between full siblings, and thus mimic the effects of shared environmental effects in twin data (Eaves, 1977; Eaves et al., 1989; Heath & Eaves, 1985; Heath et al., 1985). Likewise, even if shared environmental influences are correctly inferred, twin data alone provide no basis for attributing these effects to parental versus other shared (e.g. older sibling, neighborhood, school) influences. Finally, there are some behaviors which are likely to be especially relevant to addictive disorders for which the traditional "equal environments" assumption of twin research may be problematic, notably the differing extent to which MZ versus DZ pairs share the same peer group, a factor that may be particularly important in interpreting conclusions about initiation of substance use.

Use of Twins in Pharmacogenetic Research

For alcohol and tobacco dependence, the question of whether there are important genetic influences has been answered very convincingly, at least in the case of samples of European ancestry, and the more complex etiologic questions outlined above have assumed prominence. (For illicit drug dependence, positive findings suggesting important genetic influences are beginning to emerge, for both men and women (Kendler & Prescott, 1998; Kendler, Karkowski et al., 2000; Tsuang et al., 1996), although replication studies remain rare). One major area in which the classical twin design, and especially its twin-sibship extension, holds considerable promise is in analysis of genetic effects on "pharmacologic vulnerability"; in other words, the role of genetically determined differences in metabolic, subjective, physiologic, psychomotor and other behavioral responses to alcohol or other drug challenge, and their contribution to risk of addiction. This involves bringing genetic research into the experimental laboratory, with controlled body-weight adjusted dosing and assessment of drug effects.

The potential of such an approach is best illustrated using a concrete example from the alcohol field. Substantial genetic effects on alcohol metabolism (Martin, Perl et al., 1985) and on subjective, ataxic (body-sway) and other psychomotor responses (Martin, Oakeshott et al., 1985) have been well documented. Furthermore, there appears to be relatively little overlap, under conditions of controlled body

weight-adjusted dosing, between genetic influences on metabolism and genetic influences on other response domains (Heath & Martin, 1992). The work of Schuckit, in particular, has shown that young adult males with a family history of alcoholism, compared to controls, report lower ratings of intoxication and show a smaller increase in body-sway and smaller hormonal changes after alcohol administration, and that, at long-term follow-up, reduced reactivity to alcohol challenge was a better predictor of alcohol dependence than was family history (Schuckit & Smith, 1996). Consistent with these findings, an analysis (Heath, Madden, Bucholz et al., 1999) of Australian male twins controlled for baseline drinking history and problems, and still found that a low level of alcohol response (defined by measures of subjective intoxication and body-sway) was associated with increased probability of reporting a history of alcohol dependence at follow-up, while a high level of alcohol response was protective, with a more than 10-fold reduction in rates of alcohol dependence at follow-up in those scoring in the highest quartile on the quantitative measure of alcohol response. In a multivariate analysis that included other risk-factors, low level of response to alcohol among males had effects that were comparable in magnitude to better established risk-factors such as history of conduct disorder or major depression (Heath, Bucholz et al., 2001). Most importantly, from a genetic perspective, it was possible to show that level of alcohol response was strongly correlated with genetic risk of alcohol dependence. That is, level of response was low in those reporting a history of alcohol dependence at follow-up and in non-dependent individuals with an alcohol dependent MZ cotwin (and therefore on average at high genetic risk),whereas level of response was intermediate in non-dependent individuals with an alcohol dependent DZ cotwin (intermediate genetic risk) and highest in individuals from pairs where neither twin had a history of alcohol dependence (i.e. on average at lowest genetic risk) (Heath, Madden, Bucholz et al., 1999). Unexpectedly, no significant findings emerged in women, although subsequent analyses have suggested that genetically determined differences in alcohol metabolism may be a more important predictor of alcohol dependence risk in women from this sample (Whitfield et al., submitted)

The potential for achieving important insights from pharmacogenetic research is even greater for studies involving controlled administration of nicotine. In alcohol challenge studies, it is considered unethical to administer intoxicating doses of alcohol to alcohol-naive participants. As such, the possibility remains that uncontrolled aspects of

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Table 1

Distribution (%) of Additional Full Siblings of Twin Pairs From the Australian 1989 Twin Cohort
(Based on data reported by complete pairs and single twin responders to the diagnostic interview survey {see Heath, Howells et al., 2001 for details of sample}.)

	Number of additional full siblings										
	0	1	2	3	4	5	6	7	8	9	10 or more
MZ pairs (N = 1418 pairs)	11.1	33.2	27.5	12.4	6.9	3.7	2.2	1.0	0.6	0.6	0.4
DZ pairs (N = 2061 pairs)	10.5	27.1	27.2	15.0	8.8	4.4	2.2	2.2	0.7	0.7	0.7

baseline drinking patterns that were precursors to the development of alcohol dependence were also influencing response to alcohol. (For example, some studies may have failed to control adequately for history of exposure to intoxicating doses of alcohol). In contrast, recently developed methods for the acute administration of nicotine, for example by nasal spray (Sutherland et al., 1992), allow testing of nicotine-naive adults, and those who never progressed beyond experimentation with cigarettes. At the most basic level, testing of MZ and DZ twin pairs who are concordant for never having smoked regularly permits characterization of genetic effects on subjective, physiological and performance-based measures of nicotine response in individuals with no history of exposure to tobacco products. As illustrated using simulated data in Table 2, however, this approach can be greatly strengthened by also testing non-smoking twins from smoking discordant pairs, stratified by twin pair zygosity and presence or absence of a history of nicotine dependence in the cotwin who smoked. If genetically determined differences in level of response to nicotine at the beginning of an individual's smoking career contribute to risk of nicotine dependence, with e.g.. high level of nicotine response (Pomerleau et al., 1993) associated with heightened risk of nicotine dependence, it would be particularly informative to examine response to nicotine among non-smokers with (i) a nicotine dependent MZ cotwin, (ii) a nicotine dependent DZ cotwin, (iii) a non-dependent MZ cotwin who is a regular smoker, (iv) a non-dependent DZ cotwin who is a regular smoker, or (v) a cotwin who is also a non-smoker. The expectation is that we would observe the highest level of response to nicotine in group (i), and the lowest in group (iii), with overall ordering (i) > (ii) > (v) > (iv) > (iii). Of course, if low level of nicotine response were associated with high level of risk of nicotine dependence, the sign of these predictions would be reversed. (We assume here that being a never-smoker — that is, never having smoked a single cigarette — is not related to genetic risk of nicotine dependence in those exposed to tobacco products. The possibility that this assumption is violated, e.g. because of aversive reactions to passive tobacco exposure, must of course be acknowledged, though we consider it unlikely. Such an effect would reduce power, but should not fundamentally change patterns of response in cotwins of non-smokers.) From the perspective of alcoholism research, these same predictions will apply when our focus is on the relationship between response to nicotine (e.g. nicotine-alcohol cross-tolerance effects; Collins et al., 1996) and alcohol dependence risk.

Twins Plus Parents

Several important limitations of the classical twin design concerning assortative mating and parent-offspring environmental effects can, under certain circumstances, be overcome by extending this design to include assessment of the parents of the twins. Under certain strong assumptions, the twins plus parents (or "twins-on-the-bottom") design will allow estimation of assortative mating effects and parental environmental effects on their offspring. If these assumptions hold, the twins plus parents design indeed is probably the most powerful intact family design for resolving genetic and shared environmental effects, assortative mating effects, and parent-offspring environmental effects (Heath et al., 1985). A natural extension of the twin study is therefore assessment of the biological parents of such twins, providing a preliminary test for environmental correlations of offspring psychopathology with parental alcoholism, controlling for genetic transmission and assortative mating. The assumptions that are required however are strong, including (i) no genetic non-additivity, (ii) no genotype x age interaction effects nor other generational change in the importance of genetic and environmental influences, (iii) correct specification of the causes of spousal resemblance (Eaves et al., 1978).

The twins plus parents design has sometimes been applied in combination with measured indices of the parental environment (e.g. parental separation; Kendler et

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Table 2

Predicted Mean Differences in Nicotine Sensitivity Score as a Function of Twin Pair Zygosity and Smoking Status
(Assumes dependent and non-dependent smokers are selected from the top 25%, bottom 25%, of a quantitative measure of nicotine dependence; 60% heritability of nicotine sensitivity score; 60% heritability of nicotine dependence; one-half of the genetic variance in nicotine dependence liability is mediated through genetic influences on nicotine sensitivity. Predicted values for nicotine sensitivity scores were derived using the Pearson-Aitkin selection formula {Neale & Cardon, 1992}).

Smoking Status	Dependence Liability (not directly observable)				Nicotine Sensitivity Score			
	Twin A		Twin B		Twin A		Twin B	
	M	σ^2	M	σ^2	M	σ^2	M	σ^2
(i) MZ discordant pairs — dependent/never smoked	1.27	0.24	0.76	0.73	0.62	0.82	0.54	0.86
(ii) DZ discordant pairs — dependent/never smoked	1.27	0.24	0.38	0.93	0.62	0.82	0.27	0.97
(iii) MZ or DZ concordant never-smoked pairs	0.00	1.00	0.0	1.0	0.0	1.0	0.0	1.0
(iv) DZ discordant pairs — non-dependent/never smoked	−1.27	0.24	−0.38	0.93	−0.62	0.82	−0.27	0.97
(v) MZ discordant pairs — non-dependent/never smoked	−1.27	0.24	−0.76	0.73	−0.62	0.82	−0.54	0.86

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al., 1996) in an attempt to isolate specific sources of parent-offspring environmental influence. Unfortunately such an approach is highly dependent upon correct specification of the relationship between the parental environmental index and parental psychopathology — for example, whether parental separation is better viewed as being determined by parental alcoholism, or by associated risk-factors (e.g. parental antisociality, or some similar variable that is a genetic correlate of alcohol dependence). In the absence of data using other sampling approaches (e.g. twins and their offspring, as discussed below), no strong test for mis-specification of the model is possible, so that inferences about the presence or absence of an effect will have high probability of being erroneous.

Twins and Spouses

The causes of spousal resemblance for alcohol, tobacco or other illicit drug dependence have received relatively little research attention, notwithstanding the potential importance of this issue for understanding the long-term maintenance of substance use disorders. Incorrect specification of models for spousal resemblance (for example, attributing an observed marital correlation to reciprocal spousal environmental influences ("spousal interaction") when it is in fact due to mate selection, or vice versa) may also lead to incorrect inferences about the importance of familial environmental effects when twins-plus-parents or similar intact family designs are used for sampling (Heath & Eaves, 1985; Heath et al., 1985). Longitudinal assessment of newlyweds does not avoid this problem, since convergence of partners with respect to a variety of behaviors can plausibly be assumed to predate behaviors such as marriage. Conversely, apparent spousal convergence occurring after marriage can still be explained by assortative mating; for example, if assortation is based on parental phenotypes such as parental ability that are not manifest at the time of mate selection. As reviewed elsewhere, for a given degree of spousal resemblance, reciprocal spousal interaction will in general generate much lower correlations between the first twin and the spouse of the second twin, and vice versa, as well as between the spouses of first and second twins from a pair, than would be predicted under most plausible models of mate selection (Heath, 1987). Thus effects of assortment and reciprocal spousal influence can in principle be resolved even in cross-sectional data on twin pairs and their spouses. Different mechanisms of mate selection (for example, direct phenotypic assortment for alcoholism, versus social homogamy based on socioeconomic or other family background risk-factors) will likewise lead to different predictions for twin-cotwin's spouse and spouses of twins correlations, with only the former hypothesis predicting differences as a function of twin pair zygosity (Heath and Eaves, 1985). Assessment of twin pairs and their spouses thus allows a variety of hypotheses about the causes of spousal resemblance to be tested, minimizing the dangers of misspecification of models for spousal resemblance.

Twins-on-top Designs: Children of Twins

We may label the "children of twins" design (Cloninger et al., 1979; Heath et al., 1985; Nance & Corey, 1976), which involves assessment of adult twin pairs and their partners and biological children, as a quasi-adoption design. It allows us to compare (i) children at high genetic and high environmental risk (raised by an alcoholic biologic parent), (ii) children at low environmental risk but high genetic risk (parent is non-alcoholic but has an MZ cotwin who is alcoholic), (iii) children at low environmental but intermediate genetic risk (parent is non-alcoholic but has a DZ cotwin who is alcoholic), and (iv) control children at low genetic and low environmental risk (neither parent, nor parent's cotwin, has a history of alcoholism). Of course, since both biologic parents contribute to the genotype of their child, statistical control for assortative mating or cross-assortative mating is critical (see previous section). With appropriate control for assortative mating and comorbid psychopathology, in the absence of any environmental influence of parental alcoholism, the risk to the child of an alcoholic twin should be no higher than the risk to the child of a non-alcoholic parent who is a monozygotic cotwin of an alcoholic. Thus excess rates of alcoholism in the former group, after psychopathology in the marrying-in spouse is controlled for, implies an environmental impact of parental alcoholism. The addition of DZ twin pairs and their spouses and offspring is critical since, in the absence of this group, equally elevated rates of alcoholism in the offspring of alcoholics and of non-alcoholic MZ cotwins of alcoholics could be explained by either genetic transmission, or the environmental effects of a risk-factor for which the twin pairs were perfectly correlated (e.g., religious affiliation). Such offspring-of-twins designs are sometimes conceived of as matched-pairs designs (e.g. focusing on discordant MZ, or discordant MZ and DZ pairs). In practice, however, a between-group approach is likely to be more powerful. Among other strengths, it avoids the difficulties presented by differences in age distribution of children of two twins from a pair.

There are several critical etiologic questions that can be addressed with considerable power using the twins-on-top design. Given the practical limitations of the adoption design, the twins-on-top design should provide the most convincing evidence for environmental consequences of parental alcoholism, particularly in projects which seek to assess pertinent environmental risk-factors. There are several reasons why this is the case. First, the classical twin design provides a direct test of the hypothesis that a measure of parenting or some other aspect of the family environment (e.g. parental conflict or divorce) is genetically correlated with alcoholism risk, so that an association with offspring psychopathology could potentially be merely an indirect genetic correlation. Second, in the presence of non-random mating, inferences about the presence or absence of parent-offspring environmental effects are highly dependent upon correct specification of the mechanism of parental assortment (Eaves et al., 1989; Heath & Eaves, 1985; Heath et al., 1985). The twins-plus-spouses design which is embedded in the twins-on-top design allows a variety of different mechanisms of assortative mating (Heath et al., 1985) or spousal interaction (Heath, 1987) to be resolved. Taken together, these two advantages permit a strong test for the environmental consequences of

parental alcoholism, and the mediators of these effects, that avoids some of the dangers of model mis-specification inherent in the twins-plus-parents ("twins on the bottom") design discussed previously. Thus, if divorce is merely a secondary genetic correlate of offspring psychopathology (e.g., because of its association with parental alcoholism), after controlling for assortative and cross-assortative mating, we would expect to see no higher rates of psychopathology in the offspring of divorced twins than in the offspring of their non-divorced MZ cotwins; but if divorce (or some aspect of the environment indexed by divorce) is having a direct environmental impact on offspring psychopathology, then risk should be higher in children of divorced parents than in children of their non-divorced MZ cotwins.

The offspring-of-twins design also allows for powerful analyses of genotype x environment interaction and other risk-modifier effects, and, in this regard, has the important advantage that it generates some counter-intuitive predictions about family resemblance. Consider, for example, the association between depression in the non-alcoholic MZ cotwin of an alcoholic twin, and its association with depression or other psychopathology in his own children and his cotwin's children. Suppose there are important environmental vulnerability factors that interact with genetic predisposition to depression, such that heritability of depression is increased in those exposed to the vulnerability factor (e.g. early childhood trauma), and, as is plausible, these vulnerability factors are more common in families with alcoholic parents. Under these conditions we may predict that depression in the non-alcoholic MZ cotwin from discordant pairs will be more highly correlated with depression in the alcoholic cotwin's offspring (who will be more likely to be exposed to environmental trauma) than with depression in his own children.

The Importance of Traditional Families

While twin-family designs have many attractive features, they will never remove the need for traditional epidemiologic and clinically ascertained family study designs. For gene-mapping studies which attempt to use traditional genetic linkage approaches (e.g. affected sibship methods, or their extensions; Reich et al., 1998) to identify chromosomal locations of, and eventually identify, specific genes that contribute to alcohol dependence risk, the high prevalence of disorders such as alcohol dependence in general community samples, and predominance of mild cases, mean that the twin study will only rarely be a useful starting point. (We have noted earlier however that twin-sibship approaches have much greater potential when a quantitative outcome measure, and especially a multivariate set of quantitative phenotypes, can be defined). There are many other research questions which require families meeting unusual criteria: the additional restriction that a family include a twin would make such twin-families too rare to be useful. At the beginning of the life-span, research questions about long-term effects of maternal substance use are vulnerable to unmeasured confounding factors, if only a single pregnancy is considered for each mother. Mothers who smoke, drink heavily or use drugs during pregnancy may differ from other mothers in many respects, so that failure to control for maternal history of antisociality, hyperactive-impulsive symptoms, or other variables may cause associations with some aspects of offspring psychopathology that are in fact genetic to be misattributed to maternal substance use during pregnancy. Within-mother comparisons of offspring outcomes, for those mothers who have experienced a change in substance use between pregnancies, will provide more compelling evidence for fetal exposure effects on risk of psychopathology. Since mothers who report marked changes in levels of substance use between pregnancies are relatively rare, in practice studying the offspring of twin mothers is unlikely to be very informative for this question. However, non-twin mothers with such changes can be identified in sufficient numbers to be informative. Adequate representation of minority groups in many geographic regions will also require sampling of non-twin families. Finally, sampling of traditional families will provide a critical test of the generalizability of findings from twin-family studies, and in particular will be essential if we are to move on to the phase of family-based prevention or intervention research, guided by findings from genetic epidemiologic and other etiologic research.

Implementation

In this final section, we illustrate how we have attempted to implement a coordinated approach to dissecting the interplay of genetic and environmental risk-factors; here, we will give specific attention to alcoholism and smoking and to the three etiologic models and seven research questions that were posed with respect to alcoholism.

Alcohol dependence has increasingly become a disorder of early onset, with median onset of first alcohol dependence symptoms around 20 (Nelson et al., 1998). To address questions about mediators of genetic influence, environmental modifiers of genetic influence, and the developmental unfolding of genetic and environmental influences, we have implemented a prospective study of adolescent female twin pairs and their parents (Bucholz et al., 2000; Heath, Madden, Grant et al., 1999). This project involves a cohort-sequential sampling design in which cohorts of 13, 15, 17, and 19 year-old twins and their biological parents are assessed at two-year intervals. Two coordinated studies of male adolescent twins pairs and at least one parent have also been implemented; one of these studies has a primary focus on nicotine dependence, whereas the other is focused on alcohol dependence, with over-sampling of families with a parental history of alcoholism: led respectively by coauthors Madden and Heath). Both of these latter studies have also used a cohort-sequential sampling design, with intake assessments (across studies) at 11, 13, 15, 17, 19 and 21 years. Future assessment of siblings of these twins, and the addition of a small number of unlike-sex DZ pairs, is planned, to strengthen generalizability of findings, to take advantage of the informativeness of sibling assessments, and to increase the power of the classical twin design. These studies will also form an ideal basis for future studies of the consequences of early adolescent excessive drinking and other substance use problems. Finally, studying adolescent twin pairs will maximize power for addressing questions about how genes come to

influence alcohol dependence risk, the developmental course of alcohol use and problems, and the modifiers of these genetic effects.

For questions about genotype-environment correlation effects due to assortative mating and to parent-offspring environmental influences, three studies using offspring-of-twins designs have been implemented (led by coauthors: Jacob, True and Slutske). Two of these studies are focused on young adult and adolescent male and female offspring of alcoholic and control male twins and their cotwins and spouses, ascertained through the Vietnam-Era twin panel (Eisen et al., 1987; True et al., 1999), whereas the third is focused on the adolescent and young adult offspring of alcoholic and control female twins and their spouses, building upon prior research on the Australian twin panel older adult ("1981") cohort (Heath, Bucholz et al., 1997). Ongoing prospective studies of samples first assessed as adolescents (Cooper & Orcutt, 1977) or as college studentsm (Sher et al., 1996) allow for fine-tuning of hypotheses in these survey-based studies. Finally, we will implement a family study aimed at better analyses of ethnic differences in risk-mechanisms; in addition, ultra-high risk family environments will be oversampled to allow better characterization of environmental risk-mechanisms (led by coauthor Bucholz).

To address in the experimental laboratory questions regarding pharmacologic vulnerability, two studies, led by coauthors Sirevaag and Rohrbaugh, are examining (in young adults) genetic effects on reactivity to nicotine, and testing for possible interactions between nicotine and alcohol effects. The former study uses cardiovascular, electrophysiological and subjective rating measures, the latter advanced posturographic methods that are much more sensitive to alcohol effects than older ataxia measures. Because many participants in these studies are to be sampled from the ongoing adolescent twin studies, it will be possible to document representativeness of the final samples of challenge study participants, and to relate survey-based responses (e.g. concerning subjective reactions to first cigarette, or subjective tolerance to alcohol), in many cases obtained during adolescence, to measures obtained under controlled experimental conditions in early adulthood.

Future extensions of this research program, to address the goal of identifying specific genetic risk-factors, are designed to use Quantitative Trait locus linkage-mapping and association-mapping approaches of alcohol-related phenotypes. Plans include sampling twin-sibships already identified through our ongoing program of genetic epidemiologic research on adults (Heath, Bucholz et al., 1997; Heath, Madden, Bucholz et al., 1999; Heath, Howells et al., 2001; True et al., 1999) to complement the genemapping research program already in place (led by coauthor Madden) which is focused on smoking and nicotine dependence. Eventual collection of DNA samples from those who have participated in the prospective adolescent twin and offspring-of-twin studies, and collection of DNA from the challenge study participants, should allow characterization of the developmental effects, and genotype-environment correlation and interaction effects, associated with identified genetic risk-factors.

Conclusions

Using an example derived from addiction research, we have attempted to illustrate how coordinated family and twin-family research approaches can be used to test complex hypotheses about the interplay of genetic and environmental risk-mechanisms, in order to achieve a better integration of genetic and psychosocial research traditions. Such an integrated approach should allow for a more systematic and rigorous analysis of the interrelationship between epidemiologic risk-factors (Hawkins et al., 1992; Petraitis et al., 1995) and genetic influences on initiation and progression of psychoactive substance use and problems. For many of these questions — involving phenotypes for which multiple genetic risk-factors are likely to be important, but few have as yet been identified — the twin study design, and its many possible extensions, still offer enormous advantages.

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