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Genetic and Environmental Risks of Dependence on Alcohol, Tobacco, and Other Drugs

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The field of genetic research on addiction vulnerability, defined here as research designed to understand individual differences in risk of dependence on, or persistent harmful use of, alcohol, tobacco, or illicit drugs, offers great opportunities but also considerable challenges. Because genes have been identified that influence the metabolism of alcohol and have effects on alcohol-dependence risk (ALDH2, ADH2) or that influence the metabolism of nicotine (CYP2A6), the addiction field already provides illustration of the complexities that are to be anticipated as we identify other individual gene effects on human behavior. As reviewed elsewhere (see Crabbe, chapter 16, this volume), the ability to study drug effects in mice, rats, and other experimental organisms makes possible controlled breeding experiments that ultimately may identify the involvement of previously unsuspected genes in the regulation of responses to individual licit or illicit drugs, and also makes possible the dissection of the central nervous system effects of these drugs in ways that would not be possible in humans. Controlled dosing of humans, in drug challenge studies conducted in the human experimental laboratory, offers the possibility of identifying aspects of drug response (e.g., postalcohol "ataxia," or body sway; subjective, cardiovascular, and other physiological reactions to alcohol, nicotine, or other drugs) that are under simpler genetic control than more complex constructs such as dependence. At the same time, the challenges of understanding the complex interplay of genetic and environmental influences on behavior are particularly great for human drug use and dependence, where environmental influences may begin prenatally (through fetal alcohol effects; Streissguth et al., 1994; Yates, Cadoret, Troughton,

This work was supported by National Institutes of Health Grants AA07728, AA11224, AA11998, AA13321, CA75581, and DA/CA12854.

Stewart, & Giunta, 1998) and continue throughout the life span through parent, peer, and partner/spouse effects; where drug availability and use may vary markedly across different cultures and between different birth cohorts; and where the pertinent environmental risk factors may vary as a function of stage of progression of substance use or problems. The addictions field is thus fertile for those seeking to understand the interactions of individual genetic and environmental risk factors. In the present chapter, we highlight some of these issues that must be confronted as we attempt to understand individual gene effects on human addictive behaviors.

What Is Drug Dependence?

Drug dependence is defined in contemporary psychiatric practice (*Diagnostic and Statistical Manual of Mental Disorders* [*DSM–IV*]; American Psychiatric Association, 1994) by use of a drug class (e.g., alcohol, cannabis, or stimulants, which we refer to as *drug*) leading to the experiencing, within the same 12-month period, of at least three symptoms from a list of seven: (a) acquisition of tolerance to the drug; (b) withdrawal symptoms when drug use is reduced or stopped; (c) inability to quit or a persistent desire to reduce or stop using the drug; (d) loss of control over drug use as indicated by using much more than originally intended; (e) spending a great deal of time using the drug, getting the drug, or recovering from its effects; (f) giving up important occupational, social, or recreational activities because of use of the drug; and (g) continued use despite knowledge of the adverse physical or emotional consequences that the drug is having. Although very high interrater reliability can be achieved using standardized diagnostic interview assessments of drug dependence (e.g., Bucholz, Cadoret, et al., 1996; Hesselbrock, Eaton, Bucholz, Schuckit, & Hesselbrock, 1999), there remain unresolved questions about how best to operationalize individual diagnostic criteria for particular drug classes, or indeed about whether individual criteria are appropriate for some drug classes. Thus the existence of a cannabis withdrawal syndrome, denied in *DSM–IV*, remains controversial (e.g., Wiesbeck et al., 1996). The discrimination between tolerance to alcohol associated with central nervous system changes occurring after chronic heavy alcohol use and behavioral adaptations to the effects of alcohol in adolescents who are learning to drink, and sometimes drinking very heavily, remains beyond the resolving power of many diagnostic interviews. The extent to which specific genetic (or environmental) risk factors may differentially predispose to particular symptoms (e.g., withdrawal) in humans remains unknown, although findings in other species support this possibility (e.g., Buck, Metten, Belknap, & Crabbe, 1997). Tackling such questions, however, is likely to require a move away from reliance on current approaches to the diagnosis and assessment of substance dependence in genetic research.

Defined by contemporary diagnostic criteria, dependence on alcohol (alcoholism), tobacco, or illicit drugs (substance dependence) is widespread, with lifetime rates of dependence (i.e., the proportion of individuals reporting a history of dependence, though not necessarily currently affected) increasing in younger birth cohorts (e.g., Grant, 1997; Kessler et al., 1994; Kandel, Chen, Warner, Kessler, & Grant, 1997). Reported lifetime prevalence of *DSM–III–R* (American Psychiatric Association, 1987) alcohol dependence in the U.S. National Comorbidity Survey, for example, was 20.1% for males and 8.2% for females, with corresponding figures of 9.2% and 5.9% for illicit drug dependence (Kessler et al., 1994); and 20% lifetime prevalence of *DSM–III–R* nicotine dependence was reported for a sample of young adult men and women (Breslau, Kilbey, & Andreski, 1991). This high prevalence of dependence itself poses a challenge to genetic researchers, because the increase in risk to the full siblings of a substance-dependent proband, compared with risk in the general population, though highly significant, for *DSM–IV* dependence criteria, is of the order of only 1.5- to 2-fold, a magnitude of effect that predicts relatively poor power for use of traditional linkage methods (Risch, 1990). Substance dependence is typically of early onset, particularly in recent birth cohorts, with median age of individual alcoholism symptoms, for example, reported to be age 20 in the recent U.S. National Comorbidity Survey (Nelson, Little, Heath, & Kessler, 1996).

There has been a long history of attempts to subclassify drug dependence cases, most notably in the alcohol dependence field (Babor, 1996). Cloninger (1987), for example, proposed a framework that distinguished between an early-onset highly heritable subtype, seen predominantly in men and associated with antisocial features such as fighting, and a later onset type that was more moderately heritable, seen in women as well as men and associated with features such as guilt about drinking. Successful subtyping would facilitate the task of identifying individual genes associated with dependence vulnerability if it identified subtypes that were more strongly heritable or had more homogeneous inheritance patterns. Although apparent support has been found for such subtypes in Scandinavian data (e.g., Cloninger, Bohman, & Sigvardsson, 1981; Kendler, Karkowski, Prescott, & Pedersen, 1998), and variant classifications have been proposed (e.g., Babor et al., 1992), support for the use of such classification schemes in genetic research using diagnostic interview-based data has been scant: The Cloninger and Kendler studies used official records for analysis: Swedish Temperance Board registrations for alcohol-related offenses. The empirical basis for distinguishing high heritability and intermediate heritability subtypes in the original Stockholm adoption study also does not appear strong (Heath, Slutske, & Madden, 1997).

Latent class analysis (e.g., McCutcheon, 1987), which may be viewed as a categorical variant of factor analysis and which hypothesizes the existence of categorical rather than continuous latent variables, has been used in an attempt to define rigorous classification algorithms to subtype substance-dependent cases (e.g., Bucholz, Heath, et al., 1996; Heath et al., 1994; Kendler et al., 1998; Madden et al., 1997). Factor analysis implies the hypothesis that the observed correlations between a set of symptoms or other variables can be explained by the influence of a much smaller

number of continuously distributed latent factors; latent class analysis implies that symptom correlations can be explained by the existence of a small number of discrete, mutually exclusive classes, having class-specific symptom endorsement probabilities. Parameters of the latent class model are class membership probabilities (i.e., prevalence estimates) and class-specific symptom endorsement probabilities; and from these can be derived conditional probabilities of membership in each of the estimated latent classes, given any observed profile of symptoms. Advantages of the latent class approach are that it thus allows probabilistic classification, taking into account the fact that some cases can be assigned to a given class with probability close to unity, whereas for others membership in two or more different classes may be equally probable; and that it can, in principle, be extended to incorporate a genetic modeling approach (Eaves et al., 1993).

Figure 17.1 compares two hypothetical latent class solutions. The first (Figure 17.1a) corresponds to the case in which there are genuine subtypes, with one subtype having especially high endorsement of one subset of symptoms and the second subtype having high endorsement of a second subset of symptoms. This type of pattern is seen for symptoms of attention deficit hyperactivity disorder (ADHD; e.g., Hudziak et al., 1998; Neuman et al., 1999) and broadly supports the subclassification of ADHD into in-

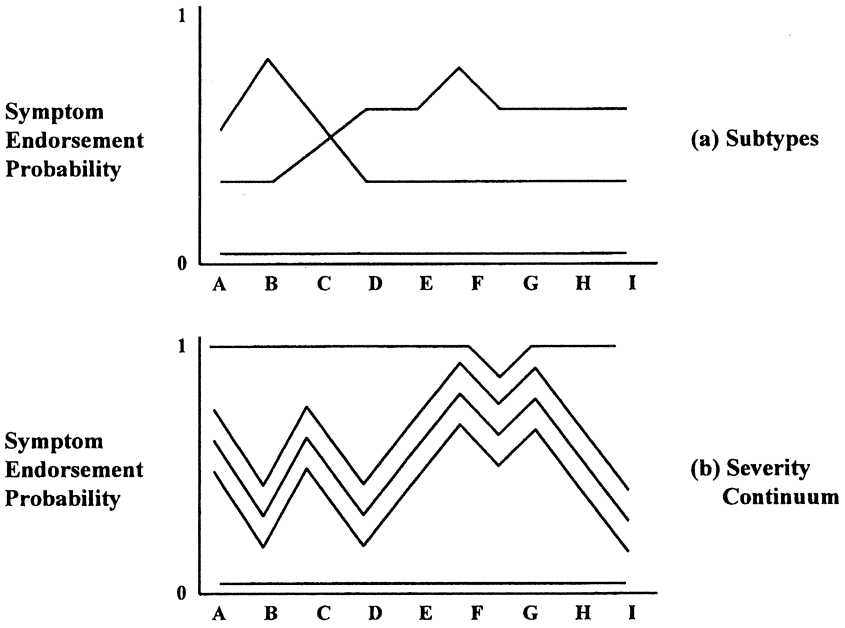

Figure 17.1. Symptom endorsement profiles for two hypothetical latent class solutions: (a) genuine subtypes and (b) cases in which classes differ in severity. Upper case letters denote different symptoms. Lines are used to show symptom endorsement probabilities for different classes.

attentive, hyperactive-impulsive, and combined types. The second hypothetical solution (Figure 17.1b) corresponds to the case in which classes differ in their severity, with symptom endorsement probabilities varying for different symptoms and also increasing from least severe to most severely affected classes, but in this idealized case with no crossing over of the symptom endorsement probability plots for the different classes. Latent class analysis has been applied most frequently in the alcohol field, although findings appear to be similar when applied to tobacco or other drug classes. Very consistently, whether applied to alcoholism symptoms reported by general community samples (e.g., female and male twins from an Australian twin study; Heath et al., 1994; Heath, Slutske, Bucholz, Madden, & Martin, 1997) or reported by severe alcoholic probands and their relatives from a family study (Bucholz, Heath, et al., 1996), it is the second pattern that is observed. Some symptoms (e.g., alcohol withdrawal, unsuccessful efforts to cut down on drinking) are endorsed with high probability by only the most severe classes. Others (e.g., alcohol tolerance, use in greater amounts than intended, persistent desire to quit or cut down) are endorsed even by relatively mild problem classes that endorse few or no other symptoms. Thus there is no evidence to support subtyping using dependence symptoms, at least in the sense implied by Figure 17.1(a).

Many traditional diagnostic distinctions are also not supported by results of these same latent class studies. Thus *DSM–IV* makes a distinction between substance *abuse* and *dependence*, with the former to be diagnosed in those not meeting criteria for dependence and sometimes considered a milder form of problems ("maladaptive pattern of substance use"; American Psychiatric Association, 1994). In the case of alcohol-related problems, with the exception of recurrent hazardous substance use (e.g., drunk driving), which in some cultures is widely reported by men even with no other problem use, other abuse symptoms are endorsed with high probability only by the more severe alcoholism classes (e.g., Bucholz et al., 1996). Likewise, the subclassification of dependence into subtypes with and without physiological dependence (American Psychiatric Association, 1994), on the basis of symptoms of either tolerance or withdrawal, is not supported by the finding that symptoms of tolerance are commonly reported by those with otherwise mild problems, while withdrawal is typically reported only by the most severely affected individuals. Finally, the use for the same diagnostic criterion of symptoms that have very different endorsement patterns—for example, a history of unsuccessful attempts to quit, and persistent desire to quit, which as we have noted above are endorsed by severe versus even moderate problem classes—also introduces considerable heterogeneity into the range of individuals who are being classified as dependent. All of these issues raise concerns about unthinking acceptance of traditional diagnostic criteria for genetic research.

The results obtained by latent class analyses of alcoholism symptoms, taking the form of Figure 17.1(b) rather than Figure 17.1(a), are consistent with two quite distinct (but not necessarily exclusive) etiologic models, which we may summarize as the *continuum* and *stage* models. Under the stage model, it is hypothesized that dependence progresses through a se-

ries of stages, from initial mild dependence progressing to severe dependence with withdrawal, with risk factors (genetic or environmental) for progression to the mildest stages not necessarily the same as risk factors for progression to the most severe stages (e.g., Bucholz, Heath, & Madden, 2000; Nelson, Heath, & Kessler, 1998). For the purposes of genetic research, the stage model would suggest that much narrower definitions of drug dependence (e.g., requiring the presence of severe symptoms such as alcohol withdrawal) will be required to discover genes that play a critical role in transition to the most severe stages of dependence. Under the continuum model, it is hypothesized that there is a continuum of severity of substance-related problems, and that it is quite possible that the same risk factors are operating throughout the range of dependence severity, implying that use of quantitative symptom count measures may be more useful than an arbitrary dichotomization into dependent and unaffected cases. More progress in clarifying these questions can be made using traditional quantitative genetic or genetic epidemiologic methods (e.g., Neale & Cardon, 1992), although the clearest insights will come from studying individual gene effects on progression of symptoms. Moving beyond the arbitrary dichotomization into dependent and unaffected cases is essential if progress is to be made in addressing such questions. For the purposes of genetic research, we still do not have convincing answers to the question: What is drug dependence?

Lessons to Be Learned From Existing Studies

Gene Effects Associated With Metabolism

The best example of how a polymorphism at a single genetic locus may lead to important differences in addiction risk is provided by the example of the ALDH2 (aldehyde dehydrogenase) locus effects on alcoholism risk, documented in a series of studies conducted by Higuchi and colleagues in Japan. It is worthwhile to consider this research in some detail, for the broader implications that it carries for studying single gene effects on human behavior, as well as its particular implications for single gene effects on human addictive behaviors.

Ethanol is converted by the enzyme alcohol dehydrogenase to the toxic metabolite acetaldehyde, which is in turn converted by the enzyme acetaldehyde dehydrogenase to acetic acid. A single point mutation in the gene for ALDH2, which is located on chromosome 12 (Hsu, Yoshida, & Mohandas, 1986), leads to an inactive enzyme. Those who are homozygous for the mutant allele (ALDH2*2/*2) or who are heterozygotes (ALDH2*1/*2) have substantially elevated blood acetaldehyde concentrations after ingestion of alcohol (Wall et al., 1997) and experience a characteristic flushing response. Compared with frequencies of 58%, 35%, and 7% for the *1/*1, *1/*2, and *2/*2 genotypes in a community control sample (N = 461), Higuchi et al. (1994) reported genotype frequencies in a large series of Japanese male alcoholics (N = 655) of 88%, 12%, and 0%, respectively, demonstrating significant reductions in risk associated with the *1/*2 and *2/*2 genotypes. Similar results have been obtained in Chinese and Taiwanese alcoholic series (Chen et al., 1999; Shen et al., 1997). Although it is commonly presumed that single gene effects on behavior are always probabilistic, the risk of alcohol dependence in ALDH2*2/*2 homozygotes is close to zero, with very few cases of individuals with this genotype who have become alcohol dependent known to alcohol researchers.

The Japanese data, as well as supporting data from other Asian populations, illustrate several important principles: (a) the multigenic determination of dependence risk and relatively modest contribution of any single gene to risk of dependence, (b) the increased power that may be obtained by considering gene effects on quantitative measures compared with dichotomous disease outcomes, (c) the importance of Genotype × Environment interaction effects, (d) the importance of identifying mediating variables that intervene between genes and the final outcome of substance dependence, (e) the fact that individual risk factors (whether genetic or environmental) may have effects specific to a particular stage in the onset and progression of substance use and dependence, and (f) the possible confounding role of population stratification effects that may complicate interpretation of an association between genotype and outcome measures. Let us consider these issues in turn.

Multigenic determination. In addition to the effects of the ALDH2 locus, significant though smaller effects of a second locus, ADH2, on alcoholism risk have also been demonstrated. Alcohol dehydrogenase (ADH), responsible for most of the conversion of alcohol to acetaldehyde, is formed by a random combination of three different subunits (alpha, beta, and gamma) that are encoded by three closely linked loci on chromosome 4 (ADH1, ADH2, and AHD3 in traditional rotation), of which only the two latter are known to be polymorphic. The low-risk ADH2*2 allele encodes the Beta2 subunit associated with faster metabolism of alcohol (i.e., leading to a faster increase in toxic acetaldehyde levels; Thomasson et al., 1991) than the Beta1 subunit associated with the ADH2*1 allele. Because ALDH2 effects on risk are much greater than those of ADH2, Higuchi et al. (1994) compared genotype frequencies in alcoholics and community controls who were ALDH2*1/*1 homozygotes, finding that the ADH2*1/*1 homozygotes were significantly more common in the alcoholic than control series (30.4% vs. 7.3%) and the ADH2*2/*2 homozygotes correspondingly less common (35.8% vs. 58.1%). This association has also been confirmed in Han Chinese (Muramatsu et al., 1995; Shen et al., 1997; Thomasson et al., 1991) and Atayal Taiwanese (Thomasson et al., 1994). Although Higuchi et al. also noted an association between ADH3 genotype and alcohol dependence risk, interpretation of this latter association is complicated by the strong linkage disequilibrium that exists between ADH2 and ADH3 loci (high positive correlation between occurrence of the ADH2*1 and ADH3*2 alleles), with more recent research failing to find an association with ADH3 once this linkage disequilibrium is allowed for (Osier et al., 1999).

The relatively modest contributions of the ALDH2 and ADH2 loci to risk of alcoholism are documented by additional data collected by Higuchi and colleagues from a small community sample. Using a questionnaire measure of problems with alcohol, Higuchi, Matsushita, Muramatsu, Murayama, and Hayashida (1996) found that only 16% of men with the high-risk ALDH2*1/*1 genotype were alcoholic by the questionnaire measure, a small but significant increased risk compared with the 10% overall prevalence of alcoholism in the sample. Among individuals with the ALDH2* 1/*1 genotype, risk was as high as 29% among those with the ADH2*1/*1 genotype or as low as 7% in those with the ADH2*2/*2 genotype. In other words, knowledge of the ALDH2 and ADH2 genotypes of Japanese males living in Japan is giving important information about differences in alcoholism risk, but not much more so than, say, knowledge of the history of smoking (a risk factor strongly correlated with alcoholism risk; Madden, Bucholz, Martin, & Heath, 2000) in males of European ancestry.

Quantitative effects. In contrast to the modest effect on risk of alcoholism, defined as a dichotomous measure, seen for the ALDH2 locus in the Higuchi et al. general community sample, a much more potent effect on simple quantitative indices of alcohol consumption is observed (Higuchi et al., 1996), with a 10-fold difference in average monthly alcohol consumption between ALDH2*1/*1 homozygotes and ALDH2*2/*2 homozygotes, and a 2.7-fold increase in consumption of the former compared with ALDH2*1/*2 heterozygotes. From the distribution of frequency of alcohol consumption reported by these authors, we may compute that, even without adjustment for unreliability of measurement, approximately one third of the total variance in alcohol consumption levels in this sample is explained by this single genetic locus. In psychological research, it is extremely rare to find individual risk factors with effects on quantitative indices of behavior of this magnitude!

Genotype × environment interaction effects. Higuchi et al. (1994) also provided what is arguably the most striking example of a Genotype × Environment interaction effect on human behavior. In comparisons of genotype frequencies in Japanese alcoholics from patient samples acquired at three times (1979, 1986, and 1992), they documented a highly significant increase in the proportion of patients who were ALDH2*1/*2 heterozygotes, from 2.5% to 8.0% and finally 13.0% (in other words, a diminishing protective effect of having a single *2 allele). Higuchi et al. suggested that this change is associated with increased social pressures for drinking with colleagues after work experienced by Japanese men. The dangers of ignoring sociocultural influences on drinking patterns, and their possible interaction with genotype, are also demonstrated by the gender differences observed by Higuchi et al. (1996) in their analysis of average monthly alcohol consumption as a function of ALDH2 genotype. They reported for their female respondents similar marked increase in levels of consumption, compared with ALDH2*2/*2 homozygotes, in *1/*1 homozygotes (a 33-fold increase) or *1/*2 heterozygotes (a 2-fold increase).

However, Japanese women with the highest risk ALDH2*1/*1 genotype were reporting alcohol consumption levels similar in magnitude to those of Japanese men with the lowest risk ALDH2*2/*2 genotype. Overall, average monthly alcohol consumption levels by Japanese women in Japan were 5–10 times lower than those of Japanese men. In contrast, among Americans of Japanese ancestry, living in a culture in which there is near universal acceptance of drinking by women, a much smaller gender difference in consumption levels is seen.

Mediating variables. Taken together, the ALDH2 genotype frequency data from the alcoholic case control series of Higuchi and colleagues, the association between the ALDH2*2 allele and the flushing response, and the associations between ALDH2 genotype and average monthly alcohol consumption suggest a very simple *mediating variable* model for the association between genotype and alcoholism risk. Impaired metabolism of alcohol associated with possession of a single ALDH2*2 allele may lead to unpleasant reactions after ingestion of alcohol, hence decreased average levels of alcohol consumption, and therefore decreased risk of alcohol dependence. While this is likely to be an oversimplification—for example, there is some suggestion that ALDH2*1/*2 heterozygotes find low doses of alcohol more pleasant, rather than less pleasant, than *1/*1 homozygotes (Wall, Thomasson, Schuckit, & Ehlers, 1992; Luczak, Elvrie-Kreis, Shea, Carr, & Wall, 2002)—it generates a model that can be tested prospectively (e.g., beginning in adolescence) to examine the role of genetic effects on consumption in accounting for genetic effects on alcoholism risk. In contrast to the association between ALDH2 and consumption levels, Higuchi et al. (1996) failed to find a significant association between ADH2 genotype and consumption levels, leading them to the conclusion that ADH2 effects must be mediated through other effects, perhaps only seen at much higher levels of alcohol consumption than would be seen in a general community sample. Establishing that a particular gene has a significant effect on risk of substance dependence still leaves unanswered many questions, including behavioral questions, about how that effect arises.

Stage-specific risk factor effects. Other studies on Japanese and other Asian populations show that while possession of a single ALDH2*2 allele on average has a protective effect on level of alcohol consumption and on alcoholism risk in those who progress to heavy drinking, presumably because of the increased exposure to acetaldehyde associated with impaired metabolism, possession of an ALDH2*2 allele becomes associated with increased risk of adverse medical consequences, including increased frequency of alcohol-related cancers (e.g., Hori, Kawano, Endo, & Yuasa, 1997; Yokoyama et al., 1996). This extreme example, in which a protective factor at one stage (the progression to heavy drinking) becomes a risk factor for a later stage (the experiencing of alcohol-related medical complications), illustrates the fundamental principle that risk factors, genetic

or environmental, may have effects that are limited to particular stages in the progression of addictive behaviors.

Population stratification effects. Compared with most Western societies, which comprise individuals of very diverse ancestral origins, Japan is considered a relatively genetically homogeneous society. This is an important consideration in the interpretation of case control data, such as the Higuchi series of alcoholics and community controls, because if there are sociocultural differences between individuals of different ancestral origins (e.g., Scandinavian Lutherans vs. Irish Roman Catholics), as well as gene frequency differences, then these sociocultural differences may be important confounding factors that can create false-positive associations, or alternatively mask associations, with individual genetic loci. In an extreme case, we may even find a genetic association for a nongenetic trait (e.g., a "Lutheran" religious affiliation gene).

The practical importance of such confounding effects (termed *population stratification effects* by geneticists) has sometimes been questioned (e.g., Morton & Collins, 1998; see also Cardon, chapter 4, this volume). However, sociocultural confounding factors are likely to be particularly important for behaviors related to alcohol, tobacco, or other drug use that show pronounced prevalence differences between different societies, or even between different age cohorts within societies. For this reason, despite the greater statistical power of traditional case control tests for single gene effects (e.g., Risch, 2000), in most studies on Western populations it is usual to include at least a validation stage that uses strategies to control for such potential confounding effects. This may be achieved by using multiple unlinked genetic markers as a "genomic control" (Devlin & Roeder, 1999; Pritchard & Rosenberg, 1999)—the principle here being that with a sufficient number of unlinked genetic markers, it should be possible to unmix a genetically heterogeneous population into more homogeneous genetic groups, in the same way that latent class analysis or other latent structure methods may be used to identify subtypes on the basis of symptom profiles, and thus determine whether genetic background is a potential confounding factor in an observed genetic association. Alternatively, it may be achieved by using within-family comparisons, such as the transmission disequilibrium test (which obtains DNA from affected cases and both biological parents and tests for significantly more frequent transmission from the heterozygous parent to affected offspring of a candidate allele—hypothetically the ALDH2*1 allele, for example—than other alleles at that locus), or its sibship extensions (Spielman & Ewens, 1998; Spielman, McGinnis, & Ewens, 1993). Such comparisons achieve perfect matching for ancestry, because full siblings, who share the same two biological parents, necessarily share the same ancestral origins, and the biological child of those parents must likewise be of the same ancestral background. All of the Japanese studies that we have cited have used standard case control methods, assuming that confounding effects are likely to be minimal in this more genetically homogeneous society. However, even within Japan, it now is apparent that there are regional differences in ALDH2 gene frequency that correlate with regional differences in alcohol consumption levels.

More limited data are available concerning the metabolism of nicotine and its involvement in genetically determined differences in smoking behavior. Preliminary reports of an association between a CYP2A6 polymorphism and smoking behavior in a sample of European ancestry (Pianezza, Sellers, & Tyndale, 1998) were found to be marred by a genotyping error (Oscarson, 2001). As in the case of loci with effects on alcohol metabolism, it does now appear that CYP2A6 is more highly polymorphic in Asian than in European ancestry populations (Oscarson, 2001), so that it is likely that findings on associations between CYP2A6 genotype, nicotine metabolism, and smoking behavior will emerge initially from Asian populations (cf. Kwon et al., 2001; Nakajima et al., 2001).

Genetic Effects in Europeans: Implications of Twin and Adoption Studies

Results from the first large-sample alcohol challenge twin study conducted using young adult Australian twins in the late 1970s (Martin, Oakeshott, et al., 1985; Martin, Perl, et al., 1985) suggest that there are also important genetic effects on alcohol metabolism in those of European ancestry. (Quantitative genetic studies of nicotine metabolism in humans, using adequately large sample sizes to detect significant genetic effects, are only just beginning, and to our knowledge similar studies of other drugs have not yet been undertaken.) On the basis of long-term follow-up of the Australian alcohol challenge sample, it appears that these differences in alcohol metabolism may be associated with differences in risk of alcohol dependence (Whitfield et al., 1998). However, the ALDH2*2 allele has not been reliably observed in individuals of solely European ancestry and thus cannot contribute to genetic risk of alcohol dependence in this group. The ADH2*2 allele is seen only at relatively low frequency (approximately 5% gene frequency), so that there have been few studies of associations with alcohol dependence in European populations. One study of Australians of European ancestry did observe the predicted decreased frequency of the ADH2*1/*2 genotype (there were no ADH2*2/*2 homozygotes observed) in alcohol-dependent men compared with controls (2.7% vs. 15.1%), but there was a nonsignificant trend in the opposite direction in women (11.1% vs. 4.6%; Whitfield et al., 1998). Furthermore, interpretation of this traditional case control comparison in men is complicated by evidence for significant population stratification effects, with the low-risk ADH2*2 allele associated particularly with English ancestry (e.g., significantly associated with a religious affiliation of Church of England, and with endorsement of Socialist social attitudes; Heath et al., 2001). Within-sibship comparisons identified five male like-sex sibpairs discordant for ADH2 genotype: In all five cases, quantitative measures of alcohol use and problems were elevated in the dizygotic (DZ) twin with the (high-risk) ADH2*1/*1 genotype compared with the heterozygous cotwin (Whitfield et al., 1998). Thus

the evidence for an ADH2 genotype effect on alcoholism in those of European ancestry is at best marginal (but see Neale et al., 1999, for stronger evidence for ADH2 effects on alcohol consumption levels). As noted earlier, the CYP2A6 locus, when properly genotyped, shows only a very low frequency variant in those of European ancestry, which greatly limits the power to detect associations with smoking behavior in these populations.

More indirect evidence that there are important genetic effects on risk of alcohol, tobacco, or other drug dependence in those of European ancestry (there are still remarkably little data on minority groups such as those of African American ancestry) is provided by a series of large-sample twin and adoption studies. Although the earliest twin studies of alcohol and tobacco use, in particular, began in the 1950s and 1960s, the most compelling evidence for genetic effects on risk of substance dependence has been from more recent research. Extensive review of the twin and adoption data on genetic contributions to substance dependence is beyond the scope of this chapter (for reviews, see, e.g., Heath, 1995a, 1995b; Heath & Madden, 1997). Here we limit ourselves to consideration of three key issues and their implications for other genetic research: (a) shared environment effects and Genotype × Environment interaction effects on dependence risk; (b) mediators of genetic influence; and (c) genetic effects on natural history or treatment or intervention response.

Shared Environment, Genotype × Environment Interaction Effects

Adoption studies have consistently found increased risk of alcoholism or other drug dependence in the offspring of alcoholic parents compared with the offspring of control pairs (for a review of individual studies, see, e.g., Heath, Slutske, & Madden, 1997). The pioneering Danish adoption study (Goodwin et al., 1974; Goodwin, Schulsinger, Knop, Mednick, & Guze, 1977) was the first to clearly demonstrate increased risk of alcoholism to the adopted-away male offspring of alcoholic biologic parents (18%) but not to the adopted-away offspring of control parents (5%). Uniquely, the study was also able to obtain interviews with some biologic offspring who had been reared by alcoholic parents who had given other children up for adoption, finding no increased risk (17%) in this admittedly small group compared with the high-risk adoptees. Thus the importance of genetic factors in the intergenerational transmission of alcoholism (at least in males) was established. Inferences about the importance of environmental effects are limited, however, by the more limited range of environmental adversity experienced in adoptive families. In most Western societies, adoptive parents are typically older and screened for the absence of major psychopathology, and are therefore unlikely to be experiencing severe alcohol or drug dependence at the time they are rearing adoptive offspring. This limitation of the adoption design is apparent from the second major Scandinavian adoption study, the Stockholm Adoption Study (e.g., Cloninger et al., 1981; Cloninger, Bohman, Sigvardsson, & Von Knorring, 1985), which noted a very low rate of alcohol-related temperance board registrations in adoptive parents (fewer than 4% of adoptive families had one or more parents with at least one temperance board registration, in contrast to approximately 14% of all Swedish males and 32% of the biological fathers of the adopted-away offspring). For the purpose of understanding intergenerational environmental transmission of risk of alcoholism or other drug dependence, the traditional adoption design may be considered far from ideal.

In the case of the twin study, at the simplest level, inference of significant genetic effects relies on the observation, in the case of quantitative traits, of higher twin pair correlations in monozygotic (MZ) pairs, who are genetically identical, than in fraternal pairs who on average share only one half of their genes in common; or, in the case of binary traits such as history of substance dependence, significantly higher risk to the MZ cotwins of affected individuals than to the DZ cotwins of affected individuals, after controlling for any overall zygosity differences in prevalence (this latter requirement, unfortunately, is often overlooked in studies that rely exclusively on twin samples ascertained through clinically affected individuals or probands). Provided that there is no evidence that MZ pairs have had more similar early trait-relevant environmental experiences than DZ pairs (except insofar as these are elicited by the behaviors of the twins themselves)—an issue that has been studied many times without finding major effects (see, e.g., Kendler, Neale, Kessler, Heath, & Eaves, 1993)—genetic influences may be inferred. Very consistently, in studies of quantitative measures of alcohol consumption (Heath, 1995b), categorical measures of alcoholism variously defined (e.g., from treatment or other official records, from interview data, or from questionnaire data; Heath, 1995a; Heath et al., 1997), categorical measures of smoking and nicotine dependence (Heath & Madden, 1997; Kendler et al., 1998; True et al., 1999), and measures of illicit drug use (e.g., Tsuang et al., 1999) or abuse or dependence (Kendler, Bulik, et al., 2000; Tsuang et al., 1996), evidence for significant genetic effects is found.

This simplicity disappears, however, when we wish to make inferences from twin data about shared environmental influences (such as parent-offspring environmental influences, neighborhood influences, school influences, shared peer influences, or other environmental influences shared by two same-age siblings growing up together), because of the complicating factors of genetic nonadditivity and Genotype × Environment interaction effects. In an ideal twin study, data would be collected on MZ and DZ twin pairs reared together and MZ and DZ twin pairs reared apart. In practice, given the very low numbers of separated twin pairs (particularly in the case of contemporary birth cohorts) and the large numbers of pairs required for testing genetic hypotheses using binary phenotypes of intermediate heritability, most twin studies of substance dependence risk have been limited to twin pairs reared together. Table 17.1 summarizes the contributions of additive and nonadditive (i.e., dominance or epistatic) genetic effects, shared and nonshared environmental effects, as well as Genotype × Environment interaction effects, to the variances and covariances of MZ and DZ twin pairs reared together and reared apart. Inter-

Table 17.1. Contributions of Additive and Nonadditive Genetic Affects, Shared and Nonshared Environment Effects, and Genotype × Environment Interaction Effects to Twin Pair Covariances and Variance

Variance/covariance	Additive genetic variance	Nonadditive genetic variance	Shared environmental variance	Additive genetic × shared environment interaction variance	Nonshared environmental variance	Additive genetic × nonshared environment interaction variance
MZ pairs reared together	1	1	1	1	0	0
DZ pairs reared together	1/2	1/4	1	1/2	0	0
MZ pairs reared apart	1	1	0	0	0	0
DZ pairs reared apart	1/2	1/4	0	0	0	0
Phenotypic variance	1	1	1	1	1	1

Note. MZ = monozygotic; DZ = dizygotic.

actions between nonadditive genetic effects and environmental effects are ignored in the table, because these effects are likely to be slight.

In a seminal paper, Jinks and Fulker (1970) showed that data from MZ and DZ twin pairs reared together and at least one twin group (ideally two groups) reared apart were sufficient to permit estimation of additive and nonadditive genetic and shared and nonshared environmental main effects (a fact that can be clearly seen from the coefficients in Table 17.1). In the absence of separated twin pairs, however, shared environmental effects and nonadditive genetic effects will be confounded, with genetic nonadditivity decreasing the DZ covariance below one half the MZ covariance, and shared environmental effects increasing the DZ covariance above one half the MZ covariance. If nonadditive genetic effects are assumed small in magnitude, then ignoring genetic nonadditivity and using only data from twin pairs reared together should yield a reasonable (albeit only approximate) estimate of the importance of genetic effects. This strategy has been used successfully by behavioral geneticists for a wide range of quantitative traits, yielding results from twin data that are in good agreement with results from adoption or other designs. It is important to recognize, however, the approximation that is being used. Thus a recent meta-analysis of twin studies of major depression (which occurs at greater than chance rates in alcoholics, i.e., is commonly comorbid with alcoholism) concluded that the shared environmental contribution to risk of depression was consistently estimated at zero, with a very narrow 95% confidence interval (0%–5%; Sullivan, Neale, & Kendler, 2000). Yet if twin pair resemblance were entirely explained by additive genetic effects, we would not expect to observe that the shared environmental variance would be consistently estimated at its lower bound of zero across a number of different studies: Because of sampling variation, we would expect to observe at least some studies with a small positive estimate for the shared environmental variance. A more plausible summary of this pattern of findings would thus be that there are both additive and nonadditive genetic influences on risk of major depression and that the possibility of shared environmental effects on risk of depression that are being masked by genetic nonadditivity cannot be excluded.

Consideration of the possibility of Genotype × Environment interaction effects, and in particular the possibility that the effects of environmental risk factors on risk of substance dependence depend on the individual's genotype, introduces an additional complication (e.g., Eaves, Last, Martin, & Jinks, 1977; Jinks & Fulker, 1970). As shown in Table 17.1, the coefficients of the interaction components are simply given by the product of the corresponding main effects. The principal implication of this, though pointed out previously, deserves emphasis. To the extent that the environmental impact of, say, parental alcoholism or drug dependence depends on offspring genotype, this Genotype × Shared Environment interaction effect will be confounded with the additive genetic main effect in data limited to MZ and DZ twin pairs reared together (they can in principle be resolved with additional data from both separated MZ and DZ twin pairs; e.g., see Heath et al., 2002). Thus, although we may safely conclude that

there are important genetic effects on risk of substance dependence, the relative balance of additive genetic main effects and Genotype × Shared Environment interaction effects remained undetermined. Given the well-established association between parental alcoholism and early trauma such as early childhood sexual abuse, which in turn predict increased risk of substance use disorders and psychopathology (e.g., Dinwiddie et al., 2000; Kendler, Bulik, et al., 2000; Nelson et al., 2002), there is reason for concern that the importance of Genotype × Environment interaction effects in the etiology of substance use disorders has received insufficient attention.

Consideration of the limitations of the traditional adoption design for resolving environmental consequences of parental substance use disorders, as well as the confounding of additive genetic and Genotype Family Environmental interaction effects, has led to a new generation of behavioral genetic studies using an offspring-of-twins design (Jacob et al., 2001; for discussion of the offspring-of-twins designs, see, e.g., Cloninger, Rice, & Reich, 1979; Heath, Kendler, Eaves, & Markell, 1985; Nance & Corey, 1976). As summarized in Table 17.2, by studying offspring of twin pairs who are concordant or discordant for alcoholism or other drug dependence and of control pairs, one can distinguish between individuals at high genetic and high environmental risk (raised by a drug-dependent biological parent), individuals at much lower environmental risk but high genetic risk (raised by nondrug-dependent parents, one of whom is the MZ cotwin of a drug-dependent twin) or intermediate genetic risk (a nondependent parent is the DZ cotwin or full sibling of a drug-dependent individual), or individuals at low genetic and low environmental risk (from control families). Most importantly, the confounding of genetic effects and Genotype × Family Environment effects is thus avoided, because offspring at high genetic risk are observed under both high-risk and low-risk environmental conditions. Thus as progress is made in the coming years in the identification of genes that contribute to risk of substance dependence, we may also anticipate increased progress in identifying environmental risk factors that may have genotype-dependent effects.

Table 17.2. Genetic and Environmental Risk to Offspring in the Offspring-of-Twins Design, as a Function of Drug Dependence History of Parent and Parent's MZ or DZ Cotwin (see Jacob et al., 2001, for further details)

		Offspring risk	
Parental status	Cotwin status	Genetic	Environmental
Drug dependent	Any	High	High
Unaffected	Drug dependent, MZ cotwin	High	Low
Unaffected	Drug dependent, DZ cotwin	Intermediate	Low
Unaffected	Unaffected	Low	Low

Note. MZ = monozygotic; DZ = dizygotic.

Mediators of Genetic Influence

The question of whether there are important genetic effects on risk of substance use disorders has been answered convincingly in the case of alcohol and tobacco dependence; and, in the case of illicit drug use and dependence, data are accumulating to suggest that important genetic effects will also be a consistent finding. To date, behavioral geneticists have been less successful in addressing the mediating-variable questions about how genetic effects on addiction vulnerability arise. Improved insights into how genetic effects lead ultimately to differences in risk of substance use disorders should help accelerate the identification of individual genes that contribute to differences in risk; and, at the same time, successful identification of genetic risk or protective factors may be expected to lead to new insights into how gene effects on risk can arise. We know, for example, that history of alcohol dependence commonly co-occurs with affective and anxiety disorders and with antisocial personality disorder (Kessler et al., 1997). To what extent can these other disorders explain the inheritance of alcohol or other drug dependence? From the work of Schuckit and Smith (1996), we know that differences in reactions to a controlled dose of alcohol, observed in young adult sons of alcoholics and controls, are predictive of later differences in risk of alcohol dependence. Do genetically determined differences in reactions to alcohol (or other drugs) play a more important role than personality or other heritable behavioral differences in predicting risk of dependence? How does the predictive power of known genetic risk or protective factors compare with that of other known risk factors? To begin to address these questions, we need to draw together the research traditions of genetics, epidemiology, and studies in the human experimental laboratory.

We have begun this integrative process using data from the Australian twin panel 1981 cohort, who were first assessed by mailed questionnaire in 1980–1982, and an overlapping sample who participated in the alcohol challenge study described previously. Diagnostic interview follow-up data were obtained from this panel over the period 1992–1994 (Heath, Madden, et al., 1999), and those who also participated in the challenge study were genotyped at the ADH2 locus (Whitfield et al., 1998). Unexpectedly, we found that male participants in the alcohol challenge study differed only in minor respects from same-age males from the entire twin panel, although female participants in the challenge study were more likely to be heavy drinkers (Heath et al., 1999). Using a simple count of the number of *DSM–III–R* alcohol dependence symptoms as the dependent variable, we fitted a series of regression models to identify significant predictors of risk. A dummy variable was used to identify individuals who had not participated in the original challenge study, and these individuals were coded as "0" on dummy variables for the challenge study and genotype measures. In this way, it was possible to combine data from challenge study participants and other individuals from the original twin panel in the same analysis (Heath et al., 2001).

Table 17.3 summarizes the major predictors that we have identified,

Table 17.3. Predictors of Log-Transformed Alcohol Dependence Symptom Count in the Australian Twin Study (see Heath et al., 2001, for further details)

Predictor	Men		Women	
	β	SE	β	SE
High alcohol sensitivity	−0.42	0.11	−0.13[a]	0.09
ADH2*2 allele	−0.39	0.15	0.14[a]	0.16
Childhood conduct disorder	0.33	0.04	0.47	0.06
History of major depression	0.23	0.04	0.16	0.02
History of regular smoking	0.23	0.03	0.27	0.02
"Other Protestant" religious affiliation	−0.19	0.04	−0.11	0.02
Not participant in alcohol challenge study	−0.13[a]	0.06	−0.14	0.05

Note. Regression coefficients estimated under a multiple logistic regression model are shown. [a]Not significant.

showing regression coefficients from a multiple linear regression analysis that included smoking history in addition to other risk and protective factors. As noted previously based on analyses of history of *DSM–III–R* alcohol dependence (Heath et al., 1999), high alcohol sensitivity in men (scoring in the top 25% of the distribution of a composite score derived from alcohol challenge study measures of subjective intoxication and post-alcohol increase in body-sway after first alcohol dose—a phenotype that has shown moderately high heritability) was associated with significantly reduced alcohol problems at long-term follow-up. Possession of a single ADH2*2 allele had a similarly strong protective effect in men. Neither of these protective factors was a significant predictor in women. History of childhood conduct disorder was the next most potent predictor in men and the most potent predictor in women, with histories of major depression and regular smoking having more modest risk-increasing effects, and having a religious affiliation of "Other Protestant" (which would include some groups with prohibitions against alcohol use) having a modest protective effect. With the exception of religious affiliation, all of these phenotypes have previously been found to show significant heritability in this sample (Bierut et al., 1999; Heath & Madden, 1997; Slutske, Heath, Dinwiddie, Madden, & Bucholz, 1998) and thus may account for a small piece of the "genetic puzzle" of alcoholism. As noted previously, however (Heath, Bucholz, et al., 1997; Heath et al., 2001), substantial residual genetic variance in alcohol dependence risk remains when these predictors are controlled for.

Genetic Effects on Natural History, Treatment/Intervention Response

Most twin and adoption studies of substance use disorders have been cross-sectional in design and have considered only lifetime occurrence of a disorder. It is clear that there are important genetic influences on who becomes dependent. To date, quite basic questions remain unanswered about (a) whether there are important genetic influences on risk of drug dependence vulnerability, once genetic effects on level of exposure (e.g., amount of alcohol consumed) are controlled for; (b) whether there are important genetic influences on risk of progression to more severe dependence in those who become dependent, or whether chance or environmental risk factors play a more important role in determining this outcome; (c) whether there are important genetic influences on probability of remission of problems without treatment or intervention; and (d) whether there are important genetic influences on probability of a favorable response to treatment.

Conclusions: Where Next?

There is robust evidence for important genetic influences on risk of alcohol or tobacco dependence, at least in those of European or Asian ancestry, and growing evidence that genes are important predictors of risk of illicit drug dependence. As the transition is made from questions about whether genetic influences are important to discovery of which genes are important and how their effects are behaviorally mediated (e.g., through effects on substance use patterns) or moderated by specific environmental risk factors (e.g., experiencing early trauma), the field of behavioral genetics faces a number of major challenges. From this brief overview of genetic effects (and potential Genotype × Environment interaction effects) on addiction vulnerability, we may draw several conclusions about priorities for future research, which we discuss below.

Quantitative Trait Approaches to Genetic Linkage Studies

Active debate continues in the human genetics research community about the relative merits of different approaches to gene mapping, with various investigators advocating genomewide linkage versus genomewide association approaches (e.g., Risch, 2000; Risch & Merikangas, 1996) versus candidate gene approaches. For a drug such as ethanol, which has many and diverse central nervous system effects (e.g., U.S. Department of Health and Human Services, 2000), a comprehensive candidate gene approach is likely to involve studying vast numbers of candidate genes and yet still miss genes with as yet unknown effects on dependence vulnerability. Although a genetic linkage strategy is unlikely to discover genes of small effect, there is a considerable advantage to being able to identify chromosomal regions likely to contain genes of relatively major effect. Some positive linkage signals are already emerging from traditional linkage studies using clinically ascertained alcohol-dependent probands and their relatives (e.g., Long et al., 1998; Reich et al., 1998), which have identified some priority chromosomal regions for follow-up genetic association studies. There is now growing recognition of the considerable potential power of QTL or variance-components linkage approaches when a quantitative

risk factor or index of risk can be defined, particularly where large sibships (e.g., Sham, Cherny, Purcell, & Hewitt, 2000) or sibships selected through an extreme-discordant sibpair (e.g., Risch & Zhang, 1995, 1996) can be identified. The feasibility of using quantitative symptom count measures or quantitative consumption indices for gene mapping studies of addiction vulnerability deserves greater consideration than it has as yet received.

Genetic Association Studies Revisited

The traditional case control study, although potentially susceptible to false-positive findings because of population stratification effects, remains the most powerful design for detecting genuine associations (e.g., Risch, 2000). Because of the specific need to uncover gene effects on treatment response, which may ultimately lead to improved tailoring of pharmacotherapies, as well as more general effects on dependence vulnerability, it is likely that treatment trials will become a major setting for genetic studies. While space limitations have not allowed us to review the somewhat chaotic literature on candidate gene associations with substance use disorders, we may note that most studies have been smaller by at least an order of magnitude than would be desirable for a complex multigenic disorder. A drawback of exclusive reliance on dependence cases ascertained through treatment settings is that a high proportion of substance-dependent individuals do not receive treatment, with the co-occurrence of other mental disorders predicting increased likelihood of treatment contacts. Thus in the U.S. National Comorbidity Survey (Kessler et al., 1996), of those reporting a history of alcohol dependence or drug dependence in the preceding 12 months, with no co-occurring mental disorder, only 19% and 26%, respectively, reported any treatment (in contrast to 29% and 47% of those who also reported mental disorder). There is therefore also great need for genotyping of substantial series of cases and controls identified through large-scale community surveys.

Toward a Molecular Epidemiology of Drug Addiction and Other Psychopathology

Uncovering effects of environmental risk factors, understanding the mediating role of psychopathology or normal behavioral variation (e.g., personality differences), and dissecting the interplay between specific genes and specific environmental risk factors can best be achieved prospectively. Case control comparisons of treatment samples and community controls, though a useful first step, have important limitations, including the following: (a) uncertainty about generalizability of findings to the general population, because a high proportion of individuals with drug dependence do not receive treatment (Kessler et al., 1996); (b) possible false-positive associations with apparent environmental risk factors or environmental moderators of genetic influence due to recall bias, with affected individuals more likely to acknowledge or perceive adverse early experiences; and (c) difficulty in reconstructing, on the basis of retrospective report, the sequence of events that includes onset of drug dependence (e.g., did onset of depression predate or follow onset of nicotine dependence?). Retrospective studies of general community samples, as well as retrospective case control studies, will always have an important role to play in genetic epidemiology. There are some risk factors that are inherently difficult to assess prospectively: An alcoholic father who is repeatedly raping his adolescent daughter or son, or abusing his partner, is unlikely to volunteer the family to participate in research. Nonetheless, for many purposes, prospective research remains the ideal for understanding risk factor and outcome associations. A particularly strong case can be made for establishing prospective studies in those of Asian ancestry, because three major genetic protective factors against alcoholism or tobacco dependence have already been identified (ALDH2, AHD2, and CYP2A6), but many basic questions about the effects of these genetic factors in the context of differing predisposing risk factors (e.g., early onset depression, childhood conduct disorder) and differing environmental exposures remain unanswered.

In contrast to many areas of medicine, prospective addiction research, and psychiatric research more broadly, must necessarily begin as pediatric research. Because onset of alcohol, tobacco, and other drug use commonly occurs by early adolescence, the conventional strategy of beginning with adult samples will inevitably lead to research that is largely retrospective in nature. Given the large sample sizes required for robust detection of genetic association, particularly when allowance is made for poststratification by ethnic origin and gender, required sample sizes are likely to be of the magnitude of 100–200,000 and higher (depending on the rarity of disorders that are to be included in the investigation). Although the logistical challenges of obtaining parental consents for participation of their children in a research project (and particularly a prospective study that will include collection of DNA for genotyping) must be faced, pediatric research has important advantages, including the feasibility of school-based assessment of most children at the earliest age groups, the feasibility of obtaining sibling and peer assessments, and the feasibility of obtaining, for at least subsamples of high-risk and random control families, teacher reports and parental assessments. The use of self-administered computer-based diagnostic interviews, while also desirable for the purpose of minimizing underreporting by adolescents, allows for economies of scale that would not be possible using conventional in-person interviewing. Studies of adolescent substance use and other psychopathology have to date tended to be either large in scale but limited to predominantly non-diagnostic assessments and only rarely designed to allow genotyping, or, if diagnostic, and particularly if involving genotyping, to be much smaller in scale. It is, however, realistic to anticipate a new generation of prospective genetic epidemiologic studies, beginning at least as young as early adolescence, that will help better define the interplay between genetic and environmental risk factors in the etiology of addictive and other psychiatric disorders as well as early onset chronic physical disorders and learning disorders.

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