MOLECULAR AND CELLULAR GENETICS OF ALCOHOL ADDICTION

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Worldwide, in societies where moderate alcohol consumption is accepted as a pleasurable pastime and even an enhancer of health and well-being, it has historically been observed that a sizable minority is unable to keep within safe limits of consumption. Such individuals may abuse alcohol or become dependent. Alcoholism is today among the most pervasive psychiatric disorders. In the United States, the lifetime prevalence of alcohol dependence, the severe form of alcoholism, is 8% to 14% (1). The ratio of alcohol dependence to abuse is 1.5:1. Individuals often maintain a pattern of alcohol abuse without dependence for many years (2). Serious drinking frequently begins in adolescence, and approximately 40% of alcoholics develop their first symptoms of addiction between the ages of 15 and 19 years (3). As discussed in this chapter, heritability studies suggest that an individual's genotype confers a particular level of vulnerability, or risk. Comparative studies across populations suggest that sociocultural factors determine differences in thresholds above which an individual is likely to go beyond social drinking and slip into abuse or addiction. Is the development of alcoholism due to a unique set of biochemical and neurobiological determinants or are the causes of addiction common to many substances? Are there preexisting behavioral traits that predispose to alcoholism? These are some of the questions addressed by this chapter.

GENETIC INFLUENCES ON ALCOHOLISM: HERITABILITY STUDIES

Alcoholism is a heterogeneous disease in which the expression of genetic vulnerability is modified by environmental factors. Some of the environmental influences are uniquely experienced by the individual (nonshared) and some are shared among different individuals within the family. Numerous studies have shown that alcoholism is familial. In the National Comorbidity Survey of 5,877 individuals, it was found that alcohol use disorders aggregate significantly in families with an odds ratio of 1.93 (4). To have an alcoholic parent is a significant risk factor for the development of the disease; children of alcoholics are five times more likely to develop alcohol-related problems than children of nonalcoholics (5). It has been shown that the transmission of the vulnerability to alcoholism from parents to their daughters is due largely or entirely to genetic factors (6).

Studies of heritability, a measure of the genetic component of variance in interindividual vulnerability, indicate that genetic influences are substantially responsible for the observed patterns of familiality. Adoption studies have shown that alcoholism in biological parents predicts alcoholism in children even when the child is reared by unrelated adoptive parents (7,8). Large, well-constructed twin studies (8–10) have demonstrated that genetic factors are important in determining vulnerability to alcoholism, particularly in the more severe forms of the disease (11).

Over the past few years, several heritability analyses have been performed using the population-based Virginia Twin Registry. A study of 1,030 U.S. Caucasian female twin pairs demonstrated that whether using narrow, intermediate, or broad definitions, the concordance for alcoholism was consistently higher in monozygotic (MZ) than dizygotic (DZ) twin pairs, and the heritability of alcoholism in women is 0.50 to 0.60 (6,9). In a study of 3,516 U.S. male twins, it was found that 0.48 to 0.58 of the variation in liability for both alcohol abuse and dependence was attributable to additive genetic factors with the remainder attributable to nonshared environmental factors, which is most accurately labeled as "other," including as it does other sources of variance such as measurement error (12). The results of these two studies were confirmed in a recent analysis of 5,091 U.S. male twins and 4,168 female twins from the same registry (13). This study also added the information that the genetic sources of variability are partially, but not

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completely, overlapping in men and women. Compared with the expected genetic correlations of 0.5 for same-sex DZ twin pairs, heritability was 0.20 to 0.24 [95% confidence interval (CI) = 4% to 45%] for opposite-sex pairs. Although the heritability value of approximately 0.5 for alcohol dependence in men and women was replicated in an analysis of 2,685 male and female twin pairs from the Australian twin registry (10), no evidence was found for sex differences in sources of genetic influence. It may be that the relatively small number of opposite sex pairs—592—in the Heath et al. (10) study compared with the larger number—1,428—in the Prescott et al. (13) study limited the power to find a difference.

GENETIC HETEROGENEITY IN ALCOHOLISM

Although there is good evidence for substantial heritability for alcoholism, individual differences in clinical presentation suggest variation in origins of vulnerability. Alcoholics vary in their drinking patterns, the severity of their symptoms, and in behavioral, physical, and psychiatric sequelae. Vulnerability may reside in personality or psychiatric traits that predispose to alcohol-seeking behavior, differential response to the effects of alcohol, or differential predisposition to addiction. Vulnerability factors found in some, but by no means all, alcoholics include attentional deficits reflected by low amplitude of the P300 event-related potential (14), anxiety reflected by the low-voltage alpha EEG trait (15), and diminished subjective response to alcohol (16).

COMORBIDITY OF ALCOHOLISM AND OTHER PSYCHIATRIC DISORDERS: GENETIC DIATHESES?

Alcohol dependence is often comorbid with other psychiatric disorders, including drug abuse, major depression (MD), anxiety disorders (ADs), and bulimia nervosa (BN), or antisocial personality disorder (ASPD) (17,18). Lifetime co-occurrence is more common among women than men and is positively associated with the persistence of alcohol dependence in both men and women (18). Population comorbidity, in which two disorders may be observed to co-occur in excess, may be due to shared causation. A strength of genetic epidemiologic studies is their ability to detect evidence of shared genetic and familial environmental causation.

Severe alcoholism, suicidality, and impulsivity tend to coexist in the same individuals, usually male. The relative risk of alcoholism is significantly increased in males with either ASPD, attention-deficit/hyperactivity disorder, or childhood conduct disorder, and there is evidence of co-inheritance of ASPD and alcoholism. Alcoholism in women is associated with anxiety and affective disorders (18) and increased neuroticism (10). These data notwithstanding, a

recent evaluation of the co-inheritance of alcoholism and other disorders revealed that the inheritance of alcoholism is remarkably distinct. Female twins from the Virginia Twin Registry were evaluated for alcoholism, MD, BN, phobia, generalized anxiety disorder (GAD), and panic. Alcoholism emerged as the one disorder with a large disease-specific genetic component: approximately 75% of the genetic variance. In addition, smaller components of the genetic liability to alcoholism also loaded onto a factor common to MD and GAD as well as a factor common to phobia, panic, and BN (19).

COMORBIDITY OF ALCOHOLISM WITH OTHER SUBSTANCE ABUSE: GENETIC DIATHESES?

Alcohol, cocaine, opiate, and tobacco (nicotine) dependency co-occur more often in the population than would be expected from their frequencies (20). This raises the possibility that there may be substance-general, as well as substance-specific, components to the heritability of alcoholism (21).

Both alcoholism and drug disorders are familial; two large studies have evaluated the familial aggregation of alcohol and drug dependence (22,23). Both studies found that relatives of drug-disorder probands across a wide range of substances, including opioids, cocaine, and cannabis, had a greater rate of drug disorders themselves than relatives of controls. However, this comorbidity occurred largely independently from cotransmission of alcoholism, suggesting that the transmission of alcoholism and other drug disorders is largely independent.

The strongest evidence of a shared, as well as a specific, addictive tendency is between alcohol and nicotine. It has long been observed that there is a relationship between smoking and alcoholism. More than 80% of alcoholics smoke cigarettes and 70% are heavy smokers, compared with 30% of the general population who smoke and 10% who smoke heavily (24). In a multivariate genetic analysis of the use of tobacco and alcohol in 774 MZ and 809 DZ male and female twin pairs from the Virginia Twin Registry, the univariate heritability of alcohol consumption was 0.60 in men and 0.47 in women, and the heritability of tobacco use was 0.49 in men and 0.51 in women. Tobacco use had a stronger loading on this shared genetic factor (0.56 in males, 0.49 in females), than alcohol consumption (0.12 in males, 0.19 in females) (25). However, the precise level of the co-inheritance is less certain than the existence of co-inheritance, and the level of genetic sharing may depend on how the phenotypes are determined. In an analysis of 2,220 MZ and 2,373 DZ U.S. male twin pairs from the National Academy of Sciences–National Research Council's World War II Twin Registry, a heavy smoking, heavy alcohol genetic factor accounted for 0.45 of the heritable

variance in heavy drinking and 0.35 of the heritable variance in heavy smoking (26), and substance-specific genetic influences contributed 0.22 of the total heritable variance in heavy smoking and 0.55 for heavy alcohol use (26). A study of 3,356 male twin pairs from the U.S. Vietnam Era Twin Registry found a substantial genetic correlation ($r = 0.68$) between alcohol and nicotine dependence. Of the total variance in risk for alcohol dependence, 0.26 was common with the genetic influence on nicotine dependence (27).

THE NEUROBIOLOGY OF ALCOHOL ADDICTION

The essential features of addiction are loss of control over consumption, compulsion to obtain the next stimulus, and continuation of abuse despite knowledge of negative health and social consequences. Tolerance and dependence are due to neuroadaptations. Processes of reward and reinforcement play their most crucial role at the start of the path to addiction, after which long-lasting or permanent neuroadaptations occur. It is likely that genetic variation in this neurobiology predisposes some individuals to a pattern of increased craving and loss of control.

Addictive substances affect a range of neurotransmitter systems in different regions of the brain. However, a pathway that appears to be crucial to the action of all addictive drugs is the mesolimbic dopamine system, which originates in the ventral tegmental area (VTA) of the midbrain and projects to the nucleus accumbens (NAC), with projections also to the limbic system and the orbitofrontal cortex (28). The amygdala, hippocampus, and medial prefrontal cortex send excitatory projections to the NAC. The mesolimbic dopamine pathway is associated with the ability to feel pleasure. Serotoninergic neurons originating in the dorsal and median raphe nuclei project to mesolimbic structures, including the VTA and NAC, and may exert inhibitory control on mesolimbic dopamine neuron activity (29) (see Chapter 95).

Alcohol, psychostimulants, opiates, and nicotine (as well as tetrahydrocannabinol and phencyclidine) exert their primary reinforcing or reward effects by releasing dopamine (DA) in the NAC. The acute reinforcing actions of psychostimulant drugs is mediated both by the blockade of DA binding to its transporter, preventing the reuptake of DA from the synaptic cleft (20), and by interaction with multiple DA receptors including D1, D2, and D3 (28). Cocaine blocks the reuptake of serotonin (5-HT) and norepinephrine in a similar fashion. A functional down-regulation of 5-HT_3 receptors in the NAC may contribute to cocaine tolerance (30), whereas chronic alcohol intake increases the sensitivity of 5-HT_3 receptors (31). Chronic cocaine and alcohol administration also disrupts the endogenous opioid system (20). Nicotine's reinforcing effect is through activation of nicotinic receptors in the VTA, ultimately leading to release of dopamine in the NA (32), but the rewarding effects are also mediated by the cholinergic and serotoninergic neurotransmitter systems. The acute reward effects of opioids are enhanced by activation of μ (and possibly also δ) receptors in the VTA.

Enhanced γ-aminobutyric acid (GABA), glutamate, dopaminergic, opioid peptide, and serotoninergic neurotransmission have been associated with acute ethanol administration, and potentially mediate some of alcohol's reinforcing effects (33). In contrast to opioids, which bind to specific receptors, ethanol appears to act on a variety of targets within the cell membrane in a less specific manner, inducing effects on neurotransmitter and neurohormone membrane receptors and receptor-gated and voltage-activated ion channels as well as modulating neurotransmitter release (34). Alterations in calcium channels may be a major component of the changes that occur in the physical dependence on ethanol (35).

GABA is the major inhibitory neurotransmitter in brain. The development of tolerance may be related to ethanol-induced adaptive changes in the $GABA_A$ receptor system. $GABA_A$ receptors are the primary site of action of benzodiazepines and barbiturates, which are used in the treatment of alcohol withdrawal symptoms.

Increasing evidence suggests that ethanol's inhibition of the glutamatergic neurotransmitter pathways, especially at the level of the postsynaptic *N*-methyl-D-aspartate (NMDA) receptor, may be an important cause of its neurotoxic effects (36). Glutamate is the major excitatory brain neurotransmitter with up to 40% of all synapses being glutamatergic (36). Inhibition of GABAergic interneurons mediated via ethanol's effect on the NMDA receptor may result in disinhibition of forebrain glutamatergic neurons that augment dopamine release (37). Changes in the NMDA receptor system may underlie intoxication and withdrawal symptoms (38) as well as "blackouts" (36). Homotarrine (Acamprosate), a structural analogue of glutamate and an NMDA-receptor antagonist, has been shown to almost double the abstinence rate in recovering alcoholics (39).

Some of the rewarding effects of ethanol result from activation of μ opioid receptors in the VTA and/or δ receptors in the NAC by the ethanol-induced release of endogenous β-endorphin from terminals of the VTA and NAC as well as release of enkephalins from intrinsic enkephalin neurons in the NAC (34). The success of the opioid antagonist naltrexone in modifying drinking behavior, controlling craving and preventing relapse in alcoholics indicates that opioid receptor-mediated mechanisms are activated by alcohol (34, 40).

The co-inheritance of nicotine and alcohol dependence (27) may relate to the finding that ethanol enhancement of DA release in the NAC appears to require activation of nicotinic receptors in the VTA. Neuronal nicotinic acetylcholine receptors are structurally related to $GABA_A$ receptors

(41). Activation of these nicotinic receptors may enhance ethanol reinforcement and voluntary intake (42).

Some studies have implicated involvement of the serotonin receptors 5-HT$_3$ and possibly 5-HT$_4$ in DA release in the NAC (31,43). 5-HT$_3$ is the only known serotonin receptor that directly gates an ion channel and hence ethanol directly potentiates the effects of serotonin at this site (44). Chronic alcohol intake increases the sensitivity of 5-HT$_3$ receptors (31) and results in enhanced GABAergic activity. The 5-HT$_3$ antagonist ondansetron has been shown to reduce alcohol consumption in alcoholics (45).

GENETIC STUDIES OF ALCOHOLISM IN HUMANS

In a heterogeneous disease such as alcoholism it is likely that there are different environment-related thresholds and different genes and gene variants. In addition there could be additive or nonadditive (epistatic) interactions between variants of multiple genes. Classic genetic analyses in rodents show that certain alcohol-related phenotypes (e.g., alcohol sensitivity) can be polygenic. On the other hand, human data showing an approximately 2:1 MZ/DZ twin ratio of concordance for alcoholism and high recurrence rates in first-degree relatives of alcoholics followed by a progressively decreasing risk in proportion to relatedness are compatible with additivity of inheritance, and do not favor multiple genes. The alcoholism genes identified so far—*ALDH2* and *ADH2*—were discovered individually but act additively when they co-occur (vide infra).

To dissect the multiple genetic influences on alcoholism vulnerability, it may be necessary or useful to consider several phenotypes representing different aspects of the disease. Attempts have been made to classify alcoholics into more homogeneous clinical groups by severity (dependence or abuse), withdrawal signs, tolerance, medical sequelae, or latent class phenotypes. Cloninger (46) divided alcoholics on a clinical and genetic epidemiologic basis into type 1 (milieu-limited, later onset) and type 2 (early onset, male dominated, associated with ASPD), thus linking premorbid personality with alcoholism vulnerability and identifying an alcoholism subtype, type II, with a stronger genetic predisposition. This classification is supported by a study involving the National Comorbidity Survey of 5,877 people in which Kendler et al. (4) demonstrated that there are two underlying dimensions of liability for alcoholism, drug disorders, ASPD, MD, and GAD that are familially transmitted with moderate specificity: (a) chronic dysphoric symptoms of anxiety and depression, and (b) acting-out behaviors and harmful substance use.

Genetics Of Alcohol Metabolism

At the present time, the genes for alcohol metabolism are the only genes that are known to have a major impact on the development of alcoholism. One gene variant (allele) is protective and the other is a vulnerability allele. Alcohol dehydrogenase (ADH) metabolizes ethanol to acetaldehyde, a toxic intermediate, which is in turn converted to acetate by aldehyde dehydrogenase (ALDH). Approximately half the population of Southeast (SE) Asian countries such as China, Japan, and Korea have functional polymorphisms at four different genes: *ADH2, ADH3, ALDH1*, and *ALDH2*. Across populations, the ALDH2-2 variant appears on a similar genetic background (haplotype) and thus has probably had the same evolutionary origin (47). The most important variants are ALDH2-2 (Glu$_{487}$–Lys$_{487}$) and ADH2-2 (Arg$_{47}$–His$_{47}$). ALDH2-2 dominantly inactivates *ALDH2*, the ALDH that is mitochondrially localized and responsible for most acetaldehyde metabolism in cells. ADH2-2 is a superactive variant. Allelic variation at ADH3 apparently exerts no independent effect on the risk for alcoholism; however, ADH3-1 is in linkage disequilibrium with ADH2-2 (48,49) and is thus also predictive of vulnerability. ADH2-2 and ALDH2-2 raise the levels of acetaldehyde by increasing the rate of synthesis, by decreasing the rate of metabolism, and by interacting additively, but not synergistically (50). The result is that ingestion of even small amounts of ethanol produces an unpleasant reaction characterized by facial flushing, headache, hypotension, palpitations, tachycardia, nausea, and vomiting (51). In an analogous fashion, disulfiram, used in the treatment of alcoholism, and some antiprotozoal drugs such as metronidazole, inhibit *ALDH2* and thereby cause a flushing reaction after alcohol consumption. Therefore, the protective effect of *ALDH2* genotypes can be regarded as analogous to protection with disulfiram, as this flushing reaction, severe in homozygotes but milder in heterozygotes, deters individuals with the protective alleles from becoming alcoholic. The allele frequency of the dominantly acting ALDH2-2 is 0.3 in Japanese and Chinese, and hence about one in two individuals experience flushing after alcohol consumption. Their risk of alcoholism is reduced about four- to tenfold. Approximately 10% of Japanese are ALDH2-2/ALDH2-2 homozygotes. Thus far, only one alcoholic ALDH2-2/ALDH2-2 homozygote has been observed across a series of studies in which several hundred alcoholics have been genotyped, and that individual is the focus of a report (52).

The genetic influence of *ALDH2* variants on alcohol consumption is modified by environment. Tu and Israel (53) found that acculturation accounted for some of the variance (7–11%) in alcohol consumption for SE Asian males born in North America, although the *ALDH2* polymorphism predicted two-thirds of the alcohol consumption and excessive alcohol use. Also, there are large differences in the prevalence of alcohol dependence in populations that have similar *ALDH2* allele frequencies. The frequencies of the *ADH2* and *ALDH2* variants are similar, but the prevalence of alcoholism is 2.9% in Taiwan and and 17.2% in

**TABLE 99.1. THE RELATIONSHIP OF *ALDH2*
AND *ADH2* GENOTYPES AND THE RISK FOR
ALCOHOLISM IN SOUTHEAST ASIANS (48)**

ALDH2	ADH2	Protective Factor
*2/*2	*2/*2, *1/*2, or *1/*1	High
*2/*1	*2/*2 or *2/*1	
	*1/*1	↓
*1/*1	*2/*2	
	*2/*1	
	*1/*1	None

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

Korea, suggesting that there are interactions with other genetic and environmental factors (54).

The *ADH2* genotype has been shown to be an independent factor contributing to the risk of alcoholism (50) and acts additively with the ALDH2-2 variant (Table 99.1). A pilot study found that the ADH2-2 allele accounts for 20% to 30% of the alcohol intake variance between two groups of light-drinking and heavy-drinking Israeli Jews, and suggests that the relatively high frequency of the ADH2-2 allele might contribute to the generally perceived lower levels of alcohol consumption and increased sensitivity to alcohol observed among Jews (55,56).

The ADH2-3 allele is a high activity variant identified in approximately 25% of African-Americans, which increases the rate of ethanol metabolism (57). In one report, the presence of ADH2-3 in African-American mothers drinking during pregnancy was associated with a lower rate of alcohol-related birth defects (58).

DETERMINING THE GENETIC BASIS OF VULNERABILITY TO ALCOHOLISM

Genetic analysis of complex disorders is complicated by the fact that any single gene is likely to account for only a small part of the variance. To detect subtle genetic effects, large samples are needed. The four methods (59) most widely used are (a) linkage analysis: the inheritance pattern of phenotypes and genotypes are elucidated in pedigrees; (b) allele sharing methods: affected relatives are compared to detect excess genotype sharing; (c) association (case-control) studies: unrelated affected and unaffected individuals are compared; (d) analysis of inbred, transgenic, and gene-knockout animals (principally mice and rats).

Linkage analysis has been successfully employed in finding major genes in diseases such as Huntington's disease, but it has limited power to detect genes of modest effect (60). An alternative approach is to employ an endophenotype as a trait specific marker, e.g., the low-voltage alpha EEG trait that is associated with alcoholism and anxiety disorders (15). Such endophenotypes may be influenced by variation at fewer genes. The brain is relatively inaccessible, so it has been more difficult to obtain biochemical and physiologic measures that identify more specific genetic subtypes as was done decades ago for certain other common, broadly defined diseases (e.g., anemia). Association studies of candidate genes, although laborious, have far greater power for untangling the genetics of complex diseases than linkage analysis (60). New approaches, including TDT (transmission disequilibrium test) analysis (61,62) and ethnic matching using informative markers (63), have been used to avoid the problems of population stratification, i.e., ethnic mismatching of cases and controls.

Rodent Models: Quantitative Trait Locus Analyses

Rodent strains are inbred to produce large numbers of genetically identical animals that can be maintained under controlled environmental conditions and intercrossed when required. The neurobiology of reinforcement and reward was elucidated largely through behavioral and anatomic studies in rodents. Several behavioral traits in rodents are continuous and polygenic. Each of the multiple genes responsible for such quantitative traits is termed a quantitative trait locus (QTL). Several QTLs may influence one trait, one QTL may affect several traits, and these QTLs can be individually mapped, with the ultimate goal being to identify genes that play a role in human addiction to alcohol. The knockout of an individual gene in the mouse can reveal a potential role for the equivalent (homologous) gene in the human.

Several QTLs for alcohol-associated behaviors have been identified in mice by using recombinant inbred strains that differ widely with respect to many alcohol-related traits, and by follow-up studies using interstrain crosses and congenics. The behaviors for which QTLs have been mapped include acute and chronic alcohol withdrawal sensitivity, alcohol consumption, and alcohol-associated hypothermia. Buck et al. (64) have shown that 68% of the genetic variability for genes influencing alcohol withdrawal severity can be assigned to QTLs on mouse chromosomes 1, 4, and 11. The locus on chromosome 11 accounted for 12% of the genetic variability in withdrawal liability and was near the genes for the γ_2, α_1, and α_6 subunits of GABA$_A$ receptors. Furthermore, a γ_2 subunit polymorphism has now been found to be genetically correlated with alcohol withdrawal severity in mice (65). QTLs for alcohol-induced hypothermia, alcohol consumption, and certain responses to amphetamine and morphine are located on chromosome 1 (66) and also on chromosome 9 near the serotonin 5-HT$_{1B}$ receptor and dopamine D2 receptor genes (67). These genetic interrelationships between different phenotypes indicate that the same genes influence different alcohol-associated behaviors, that several genes may affect one phenotype, and that some loci

for drug abuse are not drug specific. This is also evident in studies of mice in which specific genes that map to the QTL regions or candidate genes located elsewhere in the mouse genome have been knocked out. 5-HT$_{1B}$ knockout mice drink twice as much ethanol, are less intoxicated, and show enhanced aggression compared with normal mice (68). On chronic exposure to alcohol they show less evidence of tolerance. These mice also work harder to self-administer cocaine and show an increased locomotor response, behaving as if already sensitized to the drug (69).

The dopamine-related genes that have been knocked out in mice are the DRD4 dopamine receptor, which is located at the site of one of the alcohol QTLs, the D1 and D2 dopamine receptors, the dopamine transporter, and VMAT2 (the vesicular transporter). The DRD4 knockout mice appear to be supersensitive to ethanol, cocaine, and amphetamine (70). Mice lacking the D2 receptor consume less alcohol than normal mice (71). VMAT2 knockout mice have a pronounced supersensitivity to cocaine, amphetamine, and ethanol (72).

For morphine preference, three loci identified on murine chromosomes 1, 6, and 10 are apparently responsible for nearly 85% of the genetic variance in this trait (73). The μ opioid receptor gene is located at the site of the largest QTL, and this QTL also affects consumption of alcohol and cocaine (73).

QTL analysis in rodents has limitations. Frequently the result is a large genomic region of interest rather than a gene. There may be functional compensation in knockout mice during development. Mice and humans may not share the same functional variants at the same allele; for example, the ALDH2-2 allele is not even present in all human populations and is not found in mice. Another problem is that behaviors in mice cannot be freely extrapolated to humans; for example alcohol preference in mice may well be taste preference (74). However, QTL analyses in mice are useful for the identification of candidate genes and gene regions.

GENETICS OF REWARD NEUROCIRCUITS, AND NEUROCIRCUITS REGULATING IMPULSE CONTROL

Candidate Gene Approach: Case-Control Association Studies

A logical approach to understanding vulnerability to alcohol addiction is to look directly for variants in genes involved in neurotransmitter pathways implicated in ethanol use. Of particular interest is the reward pathway, incorporating serotoninergic, GABAergic, dopaminergic, opioid, and glutamatergic neurotransmission, and the largely serotoninergic impulse-control pathway. Genes for neurotransmitter metabolizing enzymes, transporters, and receptors are good candidates. Because of the complexity of causation of alco-

holism, any genetic determinants of vulnerability to alcoholism and sensitivity to alcohol's effects are likely to be subtle.

Serotonin

Serotonin is involved in behavioral inhibition and is a target for the pharmacologic treatment of alcoholism. Selective serotonin reuptake inhibitors play a limited role in modifying craving for alcohol and also modify other comorbid behaviors such as depression and anxiety.

Pathologically low levels of serotonin may contribute to impulsivity and ASPD; for example, a group of criminal, alcoholic Finns was shown to have low cerebrospinal fluid (CSF) 5-hydroxyindolacetic acid (5-HIAA), the lowest levels being found in those who had committed impulsive crimes (75). These are the alcoholics who would be most likely to have a serotonin gene variant affecting function.

Serotonin Transporter
The availability of brainstem serotonin transporter, measured by (I-123) β-CIT and single photon emission computed tomography, has been found to be significantly reduced in alcoholics, and correlated with ratings of depression and anxiety during withdrawal (76). A functional polymorphism, 5-HTTLPR, in the serotonin transporter promoter region (77) has been associated fairly consistently with anxiety/dysphoria (77,78). Several association analyses have shown that the s-allele, which reduces transcriptional efficiency, is increased in French alcoholics (79), severely affected German alcoholics (80), and early-onset, violent Finnish alcoholics with ASPD (81). However, neither linkage nor association for the s-allele was found in a family-based TDT analysis of U.S. alcoholics from the COGA (Collaborative Study on the Genetics of Alcoholism) data set, some with withdrawal symptoms (82). A Japanese association study of alcoholics with withdrawal seizures was also negative (83), and in a small study the long allele was found to be associated with reduced sensitivity to alcohol, i.e., with individuals who may be more vulnerable to developing alcoholism (84). Population stratification may be a problem with these association studies as allele frequencies have been shown to vary in European-American, African-American, and Japanese populations (85).

Serotonin-Metabolizing Enzymes
A tryptophan hydroxylase (TPH) intron variant that affects splicing is associated with reduced 5-HIAA and suicidality in impulsive alcoholics (86,87).

Serotonin Receptors
Several serotonin receptors are known to be abundant in the NAC: 5-HT$_{1B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, and 5-HT$_6$. There are as yet few published studies in which these serotonin receptors have been genotyped in humans.

Studies in rats suggest that activation of 5-HT$_{1B}$ recep-

tors in the NAC may be inhibitory on the behavioral effects of elevated mesolimbic dopamine transmission (88) by primarily modulating the activity of glutamatergic hippocampo-accumbens pathways and only secondarily altering NAC DA levels (89). In a large sib-pair linkage analysis of Finnish alcoholic criminal offenders, significant evidence of linkage and association of antisocial alcoholism to *HTR1B G861C* was found, and this was also observed in a Southwest American Indian tribe, suggesting that a locus predisposing to antisocial alcoholism may be linked to *HTR1B* at 6q13-15 (90).

Activation of 5-HT$_{2C}$ receptors inhibits DA release in the NAC (91). However, the functional Cys23Ser polymorphism does not appear to be associated with alcohol dependence (92).

5-HT$_3$ receptors may be involved in several facets of alcohol-seeking behavior, alcohol intoxication, and addiction (93); however, at the present time there are no published studies on the role of 5-HT$_3$ variants in alcoholics.

GABA Receptors

Cross-tolerance of benzodiazepines with ethanol and their effectiveness in treating alcohol withdrawal suggests that GABA$_A$ receptors play a key role in alcohol's effects. The GABA$_A$ receptor exists as a number of subtypes that are composed of combinations of at least 14 different subunits. Preliminary studies indicate that the Pro385Ser substitution in GABA$_A$α6 may play a role in benzodiazepine sensitivity (94) and may be associated with a lower level of response to the acute effects of alcohol (84). Several studies have found associations between GABA$_A$α6 and alcohol dependence (84,95) and antisocial alcoholism (96). Differences in allele frequencies between alcoholics and controls have been found in GABA$_A$α3 but not in a GABA$_A$α1 (97). There are positive (95) and negative (96) association studies for GABA$_A$β2 and alcohol dependence.

Dopamine

Dopamine is involved in arousal, reward, and motivation. Structural variants, some altering function or level of expression of gene product, have been found in the dopamine transporter (DAT) and in several dopamine receptor genes (*DRD2, DRD3,* and *DRD4*). At the present time, no role for variation in dopamine-related genes in alcoholism has been consistently demonstrated. The controversial association of a *DRD2* dopamine receptor polymorphism with alcoholism has been replicated in some case-control studies but not in numerous others (98), nor was it supported in two very large family linkage studies (99), one of which used the functionally impaired 311Cys variant. These family studies were not subject to the potential problems of ethnic stratification inherent in some of the *DRD2* case-control association studies (100). Two recently discovered

transcriptionally significant promoter polymorphisms offer promising tools for understanding the roles of *DRD2* (101) and DAT (102) in alcoholism.

Opioid Receptors

Three endogenous opioid receptors (μ, δ, and κ) are the targets of the major opioid peptides (β-endorphin, enkephalins, and dynorphins, respectively). The rewarding properties of μ- and δ-receptor ligands are brought about by activation of the mesolimbic dopamine system. Activation of κ receptors is dysphoric. Human and animal studies implicate the opioid system, particularly the μ opioid receptor, in both initial sensitivity or response to alcohol, and in the rewarding or reinforcing effects of alcohol. Subjects at high risk for alcoholism have been shown to have lower basal plasma β-endorphin levels but a more pronounced release after exposure to ethanol (103). Some studies have found associations of the gene encoding the μ opioid receptor, OPRM1, with some form of drug dependence (104). However, association and sib-pair linkage analyses of Asn40Asp, a μ opioid receptor polymorphism, in 100 U.S. Caucasians, 324 Finnish Caucasians, and 367 American Indians showed no significant association, even though the study had 80% power to detect a small to moderate effect of OPRM1 variation on alcohol dependence (105). Findings were also negative from a large study of German alcoholics (106) and a study of 891 drug- or alcohol-dependent subjects from African-American, European-American, and Hispanic origins (107).

NMDA Receptors

At the present time there are no published studies on the role of NMDA variants in alcoholism. Such studies would be of interest because of the role of NMDA receptors in reward, intoxication, and withdrawal, and potentially for the pharmacogenetics of acamprosate.

Nicotinic Receptors

Two classes of neuronal nicotinic acetylcholine receptor (nAchR) subunits (eight α and three β) have been identified (108). The most abundant receptor subtype in brain is composed of β$_2$ and α$_4$ subunits (109). Several lines of evidence, including drug preference in knockout mice (110), suggest that the nAchR β$_2$ subunit gene (*CHRNB2*) is involved in the reinforcing properties of nicotine. However, none of the *CHRNB2* variants found so far in humans has been associated with nicotine dependence (109). This gene has yet to be genotyped in alcoholics.

Whole Genome Linkage Scans

The power of the genetic linkage analysis approach has been greatly improved by the recent collection of very large, care-

fully phenotyped, family and population data sets such as that of COGA, a multicenter, family genetic study and the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Southwestern Indian family sample. Two studies utilizing these data sets have detected evidence of linkage of alcoholism to several chromosomal regions, with some convergent findings (111,112). In the Southwestern American Indian population isolate, suggestive evidence was found for linkage of alcohol dependence to the ADH region on chromosome 4q and to two regions harboring neurogenetic candidate genes. Those locations were chromosome 11p, in close proximity to the *DRD4* dopamine receptor and tyrosine hydroxylase gene (the rate-limiting enzyme in dopamine biosynthesis), and chromosome 4p, near a GABA receptor gene cluster (111). In the COGA families, which derive from the cosmopolitan, diverse population of the United States, modest evidence was also found of linkage to the ADH region on 4q. There was also evidence of linkage to chromosomes 1 and 7, and to chromosome 2 at the location of the opioid receptor gene (112). In addition, there was evidence of linkage of the P300 event-related potential alcoholism-associated trait to chromosome 6q in the region of a glutamate receptor (GRIK2), and to chromosome 2q near the location of two acetylcholine receptors (113).

DISCUSSION

There is abundant evidence of substantial heritability (0.5 to 0.6) of both broad and narrow definitions of alcoholism in men and women. Although the quantitative role of genetic risk factors is approximately equal in both sexes, the lower concordance of opposite-sex pairs suggests some gender-specific action of genes.

Genetic vulnerability to alcoholism may originate in personality or psychiatric traits that predispose to alcohol-seeking behavior, differential response to the effects of alcohol, or differential predisposition to addiction. Studies of the co-inheritance of alcoholism and other psychiatric disorders are beginning to emerge. There is evidence of co-inheritance of ASPD and alcoholism in men. Although alcoholism is associated with anxiety and affective disorders in women, it has been shown that 75% of the genetic variance of alcoholism is disease specific. Alcohol, nicotine, and other substance abuse disorders have been noted to co-occur, yet recent studies have shown that the transmission of alcoholism is largely independent of that of other drugs of abuse with the exception of nicotine, with which there is a substantial (0.68) genetic correlation.

The mesolimbic dopamine system is fundamental to the neurobiology of addiction. We are only at the very beginning stages of understanding the complexities of ethanol's interactions with this system. Enhanced GABA, glutamate, dopaminergic, opioid peptide, and serotoninergic neuro-

transmission have been associated with acute ethanol administration and potentially mediate some of alcohol's reinforcing effects. Genetic variants in the serotoninergic system—5-HT$_{1B}$, TPH, and possibly 5-HTTLPR—have been associated with alcoholism, particularly in males with antisocial, impulsive features. Several studies have found associations between GABA$_A\alpha 6$ and alcohol dependence. There have been no conclusive genetic findings in the dopaminergic or opioid systems to date.

Future studies are likely to focus on finding genetic variants in the neuroreceptors and ion channels that have been demonstrated to be affected by ethanol, including GABA$_A$ receptors, NMDA receptors, non-NMDA glutamate receptors, 5-HT$_3$ receptors, voltage-gated calcium channels, and neuronal nACh receptors. Of particular interest will be functional genetic variants that are directly capable of altering reward, tolerance, and withdrawal, thereby predisposing individuals to addiction to alcohol.

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