Perspectives

Biological Markers for Increased Risk of Alcoholism and for Quantitation of Alcohol Consumption

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Research over the past two decades has demonstrated convincingly that there is a genetic predisposition to alcoholism (1). Alcoholism clearly shows familial clustering (2, 3), which at present has been shown to result from both inherited and environmental factors. In studies of adopted-out children of alcoholics in Sweden, two forms of alcoholism were identified. Type I alcoholism occurs in both men and women, is not usually very severe, and is associated with similar mild alcoholism which has developed in adulthood in either biological parent. It is referred to as "milieu-limited" alcoholism because both a history of alcoholism in the biological parent(s) and environmental factors are required for the development of abnormal drinking behavior in the children. A far greater risk of alcoholism was observed in the adopted-out sons of a second group of male alcoholics. These men, suffering from what was termed type II alcoholism (1–3), exhibited early onset of drinking problems (i.e., in their teens) associated with risk-taking behavior, petty criminality, binge drinking, and drug abuse. The risk of alcoholism in the sons of type II alcoholics is about nine times the risk for males in the general population. Type II alcoholism ("male-limited" alcoholism) may account for about 25% of male alcoholics.

These findings have stimulated a search for biochemical, neurophysiological, and ultimately genetic differences between alcoholics (especially type II men and their sons) and nonalcoholic control subjects. Studies on these subjects have centered on the measurement of phenotypic markers such as serum proteins, enzymes of circulating blood cells, and electroencephalographic studies (4). Several differences have been found between the alcoholics or sons of alcoholics and controls; these differences are described below and are in varying stages of evaluation. It is not yet known if any of the available markers are pathophysiologically related to abnormal alcohol-seeking behavior or if they predict high risk for alcoholism.

The acute alcohol-flushing reaction in Asians, in contrast, has been biochemically and genetically explained as the result of a single base mutation, and the reaction is clearly a protective factor against heavy drinking and alcoholism. In fact, it is the single best-characterized genetic factor influencing alcohol drinking behavior. Whether acute alcohol-flushing reactions

or other between-individual differences in response to alcohol might influence alcohol consumption in other populations is not known at present. In addition to tests for risk of alcoholism, significant progress has been made in finding tests that can be used to detect excessive alcohol consumption. Blood tests that can reflect quantity of alcohol use are being investigated and some of them are analogous to the measurement of blood content of hemoglobin A_{1c} as an index of hyperglycemia integrated over time. These tests may have a major impact in the early screening and diagnosis of alcohol abuse and in the monitoring of response to treatment.

Biochemical and electrophysiological characteristics of alcoholics and children of alcoholics

Platelet monoamine oxidase activity. Monoamine oxidase (MAO,[1] E.C.1.4.3.4) catalyzes the degradation of monoamine neurotransmitters (norepinephrine, dopamine, and serotonin). It exists in two forms: MAO A and MAO B (5). The cDNAs for these enzymes have been cloned and the two forms are different gene products (6). The genes have been assigned to the X chromosome (7). Both enzyme forms are present in the central nervous system: MAO A has been localized to catecholaminergic neurons; MAO B is found in neurons of serotonergic pathways (8). MAO B is also found in circulating platelets (5). The activity of the platelet enzyme is genetically determined (5, 9), and variation is due to differences in the amount of enzyme rather than the K_m for substrate. Platelet MAO activity has been studied in a number of psychiatric disorders. In alcoholism, the majority of studies have found the activity to be lower in alcoholics than in controls, although methodological problems such as isolation of platelets and choice of substrate and substrate concentration for the assay confuse the issue somewhat (10–14). This difference is exaggerated if the alcoholics are stratified into types I and II. The activity in type II alcoholics is 25–30% lower than that in type I alcoholics or controls (11). The low activity persists with abstinence, suggesting that the low activity is not simply a toxic effect of ethanol on the bone marrow or platelets. The significance of this finding, which has been confirmed in a large number of independent studies, is not clear. Brain MAO has been studied in alcoholics who committed suicide and was found to be lower than in suicides in nonalcoholics and in patients dying from other medical problems (15). Since the neurotransmitter substrates of MAO are involved in mood and possibly brain reward systems, the discovered differences in platelet and possibly brain MAO might have pathophysiologi-

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1. *Abbreviations used in this paper:* ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CDT, carbohydrate-deficient transferrin; MAO, monoamine oxidase.

cal significance. However, neurochemical studies using selectively bred lines of rats that exhibit disparate alcohol consumption such as the P (alcohol-preferring) and NP (alcohol-nonpreferring) rats have suggested that the alcohol-preferring animals have lower serotonin and dopamine content in some brain regions, which would not be predicted if lowered MAO activity played a direct role in the alcohol-seeking behavior of humans (16). No prospective studies on the risk of developing alcoholism in persons with low vs. normal platelet MAO activities have been reported.

Platelet and lymphocyte adenylate cyclase activity. A second enzyme that has been found to have different activity in alcoholics is adenylate cyclase. The accumulation of cAMP in lymphocytes and platelets stimulated with a number of ligands has been found to be reduced in cells obtained from alcoholics. The effect in lymphocytes has been studied using adenosine A2 receptor agonists. Basal and adenosine agonist-stimulated cAMP accumulation in peripheral lymphocytes from alcoholic patients (unfortunately not characterized as type I or II) was reduced by about 75% (17). Normal lymphocytes exhibit an augmentation of adenosine agonist-stimulated cAMP levels when acutely exposed to ethanol; this response becomes attenuated after culturing the lymphocytes in the presence of ethanol. Similarly, lymphocytes from actively drinking alcoholics showed an attenuated response to the adenosine agonist plus ethanol. When lymphocytes from type II alcoholics were cultured in the absence of ethanol for 7–8 d, the lymphocytes not only recover responsiveness to A2 agonists but become supersensitive (18). They are also more sensitive to inhibition of adenosine-stimulated cAMP production after chronic exposure (24 h) to ethanol. It is not yet known whether this difference in lymphocyte cAMP metabolism between alcoholics and controls is genetically controlled or an effect of chronic ethanol consumption, but it may possibly help to distinguish alcoholics from others in the general population. It would be very interesting to study this phenomenon in abstaining alcoholics and their children. In addition, the accumulation of cAMP in platelets exposed to cesium fluoride, prostaglandin E, and a nonhydrolyzable analogue of GTP was decreased in cells obtained from alcoholics compared with those from controls (19). This abnormality persisted in abstinent alcoholics. All of these individuals were males, but were not subtyped as to their genetic background. The finding that the activity of adenylate cyclase is reduced when stimulated by a variety of receptor ligands and a nonhydrolyzable GTP analogue in both lymphocytes and platelets suggests the possibility that the real abnormality is in the G_s guanine nucleotide-binding protein which couples receptor binding of ligand to adenylate cyclase activation. This is under investigation.

Electroencephalographic studies. Electrophysiological studies of alcoholics and their offspring have shown reproducible abnormalities. Auditory evoked potentials are slowed in chronic alcoholics, possibly reflecting demyelination in the brainstem (20). These responses improve toward normal with abstinence, implying that this is a result of alcohol use and not an antecedent abnormality. Of greater interest is the finding by several groups that event-related potentials in males at risk for alcoholism, that is, whose fathers had an exclusive diagnosis of alcoholism (mainly type II alcoholics), are different from those of control males (21, 22). Both visual and auditory event-related potentials have been studied, and these electrical signals are thought to reflect cognitive processing of sensory input. The voltage of the P3 component (the third positive wave) was reduced in sons of alcoholics. This effect was demonstrated in boys between the ages of 7 and 13 yr, before they had been exposed to alcohol; it was not necessary to administer alcohol before the test to elicit this difference (21). These results suggest that the children of alcoholics may have a subtle abnormality in their brains, although this difference in P3 voltage has not yet been shown to predispose the children to alcoholism in longitudinal studies.

Alcohol metabolism and risk of alcoholism

The enzymes of alcohol metabolism have been the focus of a great deal of study. These are shown schematically in Fig. 1. Starch gel electrophoresis of human liver extracts reveals a complex pattern of alcohol (ADH) and aldehyde dehydrogenase (ALDH) isoenzymes (23). The human ADH complex is located on the long arm of chromosome 4, where five closely related ADH genes are found, presumedly the result of gene duplication in the past. Both the *ADH2* and *ADH3* genes are polymorphic, and three known alleles at the *ADH2* locus encode alleloenzymes with widely differing kinetic behaviors. To date it has not been possible to correlate ADH genotype with the known threefold variation between individuals in rates of alcohol metabolism, but this problem has been overcome by the recent development of methods to genotype individuals using leukocyte DNA (24). One extremely interesting but quite preliminary finding is that the ADH2[3] allele encoding

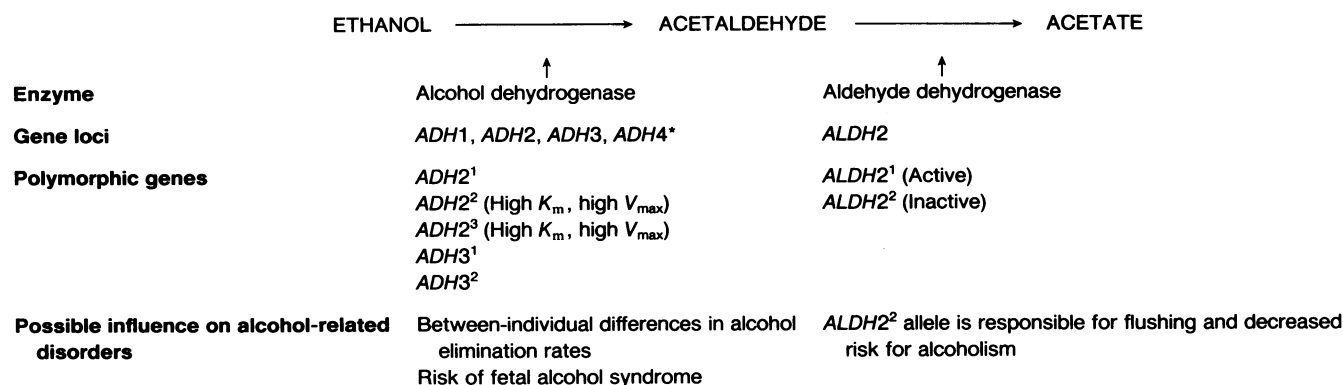|	ETHANOL $\longrightarrow$ ACETALDEHYDE $\longrightarrow$ ACETATE	
Enzyme	Alcohol dehydrogenase	Aldehyde dehydrogenase
Gene loci	ADH1, ADH2, ADH3, ADH4*	ALDH2
Polymorphic genes	ADH2[1] ADH2[2] (High K_m, high V_{max}) ADH2[3] (High K_m, high V_{max}) ADH3[1] ADH3[2]	ALDH2[1] (Active) ALDH2[2] (Inactive)
Possible influence on alcohol-related disorders	Between-individual differences in alcohol elimination rates Risk of fetal alcohol syndrome	ALDH2[2] allele is responsible for flushing and decreased risk for alcoholism

Figure 1. The pathway of alcohol metabolism by alcohol (ADH) and aldehyde (ALDH) dehydrogenases. Polymorphic gene loci and the biological effect, if any, of these variations are shown and potential effects of inheriting different genes is indicated. *Another alcohol dehydrogenase gene, *ADH5*, exists but the enzyme encoded by it does not participate in alcohol metabolism.

the $\beta3$ enzyme is unexpectedly common in a small number of Black children with the fetal alcohol syndrome (25). The $\beta3$ enzyme has a very high K_m for ethanol but has a high V_{max}, both of which suggest it may be especially active at high blood alcohol concentrations; to date it has been identified only in Blacks. If this finding can be replicated, it will be the first clear association of a particular ADH genotype with a disease state that will offer an opportunity for genetic counseling regarding an alcohol-related disorder.

One biochemical difference between alcoholics and nonalcoholics in Asians is well understood and appears to be pathophysiologically important. It has been long recognized that a substantial fraction of Asians (40–50%) experience facial flushing, tachycardia, sweating, and nausea when they drink. This alcohol-flush reaction has been shown to result from the lack of mitochondrial aldehyde dehydrogenase (ALDH2) activity (26). This was first demonstrated using hair root samples and has been confirmed in assays of liver extracts. When these individuals drink, their blood acetaldehyde rises to 10 or more times higher levels than in nonflushing individuals; acetaldehyde is believed to cause the release of vasoactive compounds from mast cells which then cause vasodilation. The acetaldehyde is ultimately oxidized by other ALDH isoenzymes with higher K_m present in many tissues. Ingestion of alcohol while taking disulfiram, an inhibitor of ALDH, is well known to cause a similar reaction. The inactive ALDH2 subunit contains a single substitution of lysine for glutamate at residue 487 (27). Our laboratory and others have developed methods to genotype this locus using allele-specific oligonucleotides to probe Southern blots (28) or polymerase chain reaction amplification products (29). We reported the unexpected finding that the mutant allele (ALDH2^2) is dominant over the normal allele (ALDH2^1), that is, heterozygotes lack enzyme activity (29). Presumedly, the incorporation of a single mutant polypeptide into the ALDH tetramer inactivates the enzyme substantially, although this awaits experimental proof. Of great interest is the finding that individuals with the inactive enzyme have a greatly reduced risk of developing alcoholism. In Japan, about 45% of the population is ALDH2 deficient; however, only 5% of Japanese alcoholics or individuals with alcoholic liver disease lack the enzyme (30–32). We have found very similar results in Chinese living in Taiwan (Thomasson, H. R., et al., unpublished results). In addition, the aboriginal people of Taiwan have a greater alcohol abuse problem and a lower incidence of ALDH2 deficiency. These studies show the ALDH2 deficiency to be the strongest genetic determinant of risk of alcoholism, or conversely, that the normal ALDH2^1 genotype is permissive of heavy drinking and alcoholism (33). The ALDH2^2 allele and ALDH2 deficiency have not been observed in Caucasians and only rarely in North American Indians (34). We and others are now examining Caucasians who experience flushing when they drink (reported to be 5–10% of the Caucasian population [35]) for the possibility that they have a different variant form of ALDH2, which in turn might alter their risk for alcoholism.

Markers of alcohol use and abuse

A major need in the field of alcohol treatment is a test to detect excessive ethanol consumption (for the early diagnosis of alcoholism) and to monitor the compliance of patients during treatment for alcoholism. Such a test should distinguish between moderate ("social") drinking and heavy drinking, should integrate the intake of ethanol over time, and prefera-

bly should be directly related to the presence of ethanol in the body and not to a response of the body to ethanol. Examples of the latter are the activity of γ-glutamyltranspeptidase, mitochondrial glutamate-oxaloacetate transaminase, and the mean corpuscular volume of erythrocytes (36). Generally the tests that reflect the development of medical complications of alcoholism are insufficiently sensitive and relatively slow to show abnormal conditions and, therefore, are not particularly useful for detecting occult alcohol abuse; definitely these are poor tests to quantify the amount of alcohol consumed over time and often give abnormal findings in non–alcohol-related diseases (37). In fact, two brief questionnaires (the CAGE test and a trauma questionnaire) are probably more sensitive than any single laboratory test for detecting alcohol abuse (38, 39). Two tests under evaluation that may circumvent these problems are assays for protein-acetaldehyde adducts and carbohydrate-deficient transferrin.

Acetaldehyde is a chemically reactive compound which can form Schiff bases with amino-terminal and lysyl side-chain amino groups of proteins, as well as other less well-characterized adducts. To date, adducts have been detected with tubulin (40), hemoglobin (41), serum proteins (42), cytochrome P450IIE1 (43), and an as yet incompletely characterized 37-kD liver cytosolic protein (44). Adducts with hemoglobin, cytochrome P450IIE1, and the 37-kD liver protein have been shown to form in alcoholic patients and animals during alcohol consumption, indicating that the low concentrations of acetaldehyde which are found in vivo during alcohol metabolism are sufficient to modify proteins. These are detectable with polyclonal or monoclonal antibodies which appear to recognize an epitope containing the acetaldehyde (44, 45). Hemoglobin-acetaldehyde adducts and serum protein-acetaldehyde adducts have been detected in venous blood of alcoholics who have been drinking recently (46); the sensitivity and specificity of these tests in detecting recent alcohol use are under study.

A test that has been characterized in greater detail is the alkaline isoform of transferrin or carbohydrate-deficient transferrin (CDT). This was initially detected by isoelectric focusing of serum from alcoholics and seen as a band migrating with a pI alkaline to that of the usual transferrin isoforms (47). This transferrin variant appears to lack terminal sialic acid residues, but the mechanism by which alcohol consumption increases its concentration is unknown. It has been found only rarely in controls (1% of a group of 100 normal individuals [48]) and was positive in 81% of individuals with a consumption of at least 60 g of ethanol per day in two different studies (48, 49). The test is not abnormal in liver diseases, with the exception of about half of nondrinking patients with primary biliary cirrhosis (49). If primary biliary cirrhosis is excluded, a positive CDT has a 99% specificity and predictive value for high alcohol consumption. The test remains positive for about 10 d after cessation of drinking, so it might be a useful test for following abstinence during treatment. Unfortunately, owing to technical problems, the CDT test is still investigational in a limited number of research laboratories and it is not ready for large-scale study.

Conclusions

The recent studies aimed at finding markers of increased risk of alcoholism are promising and merit further exploration. In addition to phenotypic trait markers, genetic markers should be sought and segregation and linkage studies should be per-

formed. The recent successes in similar studies of manic-depressive illness and Huntington's disease are encouraging to the alcoholism research community. Given what is now known about the genetics of alcoholism, these new studies should incorporate the following suggestions for experimental design. Subtyping of alcoholics as type I, type II, or nonfamilial alcoholics is needed. The number of affected siblings, parents, and grandparents should be ascertained. Maternal use of alcohol during pregnancy should be determined if possible. Most studies will probably focus on type II alcoholics because of the strong genetic influence, but this must be clearly stated. Any abnormalities found will need to be verified in different racial populations and in both sexes. Ideally, the tests should be performed on individuals who have a primary diagnosis of alcoholism, and do not have coexistent psychopathology or medical complications of alcohol abuse. The abnormalities should persist during abstinence. Ultimately, it will be necessary to determine whether the abnormality is present in children or young adults before they begin drinking, and whether the risk of developing alcoholism is increased in those with the trait. In addition, it is necessary to show that the trait is rare in the general population, is genetically transmitted, and can be reliably measured. Although none of the presently utilized tests have been validated to this extent, this should be possible in the next decade.

Validation of tests of alcohol consumption is considerably easier, but may be prone to error owing to the unreliability of reported alcohol drinking. These tests will probably be of considerable value in screening programs for alcohol abuse, for instance in industry, for monitoring compliance in alcohol treatment, and in detecting alcohol abuse as the etiology of certain medical complications, such as pancreatitis and seizures. Of course, the specificity of the tests will need to be very carefully evaluated before they can be used for nonmedical purposes, for instance to screen job or insurance applicants.

It is clear that substantial biological evidence now supports the genetic evidence for heritability of alcohol abuse. Besides serving as useful tests for advising children of alcoholics of their risk of alcoholism, the abnormalities that are being identified should give us leads to important pathophysiological bases of alcohol drinking behavior and ultimately to better forms of prevention and therapy.

Acknowledgments

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