0145-6008/05/2907-1321$03.00/0
Alcoholism: Clinical and Experimental Research

Vol. 29, No. 7
July 2005

Recent Developments in the Genetics of Alcohol-Related Phenotypes

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This article presents the proceedings of a symposia held at the International Society for Biomedical Research on Alcoholism Congress in Mannheim, Germany, in October, 2004 and focused on recent developments in alcohol-related phenotypes from three different research groups. The first presentation focused on the possible contribution of polymorphisms of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase-2 (ALDH2) as contributors to alcohol-related organ damage. As polymorphisms of both ADH and ALDH, these genes may explain individual differences in the concentration and elimination of ethanol and acetaldehyde in the blood after heavy drinking; they may also be useful for determining their possible contribution to possible alcohol-related types of organ damage, including amnestic problems and polyneuropathy. A second presentation examined externalizing behavior phenotypes, including conduct disorder symptoms, aggression, and suicidal behavior, which are all prevalent among individuals with alcohol dependence. As part of the Collaborative Study on the Genetics of Alcoholism, a genome screen was performed in multiplex alcohol-dependent families to identify chromosomal regions related to these three types of externalizing behaviors. Both the quantitative and qualitative phenotypes were examined, and evidence of linkage was found for several chromosomal regions. An area of chromosome 2 demonstrated linkages for suicidal behavior, conduct problems, and alcohol dependence, suggesting a possible sharing of genes for different externalizing behavior phenotypes. The last presentation focused on serotonin (5-HT) as being a key neurotransmitter in antisocial alcoholism and related phenotypes. In a study of adult alcoholics, an association was found between a lower frequency of the 5-HT 1B 861C allele, antisocial personality traits, and conduct disorder in alcohol dependence. Adult antisocial personality was more often found in male subjects. Based on the presented analyses, inconsistent but encouraging results were found to support the role of the 5-HT 1B G 861 C polymorphism and antisocial behavior in alcohol dependence.

Key Words: Phenotypes, Genetics, Susceptibility, ADH/ALDH, Conduct disorder, Externalizing behavior.

ALTHOUGH ALCOHOL USE behavior and alcohol use disorders are highly familial, the identification of genes that specifically contribute to the susceptibility for alcohol dependence has been difficult, and the significance of the genetic findings have often varied according to the population studies or the diagnostic criteria used to identify the alcohol dependence phenotype. Given that alcohol dependence is a complex behavior/trait and that there is considerable variation in its expression even in the general population, the lack of findings for specific genes is not surprising. Consequently, many investigators have begun to examine alcohol dependence in relation to its component parts to better understand the genetic bases of alcohol use and its consequences, including alcohol dependence. Examples of phenotypes have examined maximum drinks consumed, withdrawal behavior, and associated comorbid psychiatric conditions. Possible endophenotypes based on the subjective response to ethanol and externalizing behaviors may also provide clues to the genetic puzzle. This symposium provided new data in relation to alcohol-related clinical phenotypes to help further our understanding of genetic factors that may modify a person's susceptibility for development of alcoholism.

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Received for publication January 27, 2005; accepted March 29, 2005.

This work was supported in part by PHS grants P50-AA03510 (VH) and U10AA08403 (VH).

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DOI: 10.1097/01.ALC.0000171898.31265.52

GENETIC VARIATIONS OF ETHANOL-METABOLIZING ENZYMES, ETHANOL PHARMACOKINETICS, AND ALCOHOL-RELATED PHENOTYPES

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Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase-2 (ALDH2) play central roles in the metabolism of ethanol and its metabolite, acetaldehyde, in the liver. Genetic polymorphisms with physiological relevance are

known to exist for both ADH1B and ALDH2 loci. Since different alleles of these genes may explain individual differences in the concentration and elimination of ethanol and acetaldehyde in the blood after drinking, they may provide useful models to elucidate the contribution of these substances to the development of alcohol dependence and possibly alcohol-related types of organ damage.

First, we evaluated the pharmacokinetics of ethanol in individuals with different ADH1B and ALDH2 genotypes, using an alcohol clamping method developed by O'Connor et al. (1998). Using this technique, we measured the ADH1B- and ALDH2-associated alcohol elimination rates (AER) and examined the associated profile of blood acetaldehyde concentration (BAAC) in a sample of healthy Japanese adult volunteers.

The results are summarized as follows: 1) although male subjects had higher AERs than female subjects, this difference disappeared when the AER was adjusted by lean body mass (LBW); 2) male subjects but not female subjects with the heterozygous *ALDH2*1/*2* genotype showed significantly lower AERs than those with the homozygous *ALDH2*1/*1*; 3) unlike previous reports, our results suggested no significant difference in LBW-adjusted AER among *ADH1B* genotypes for either male or female subjects; 4) although the blood alcohol concentration (BAC) remained at a constant level, the blood acetaldehyde concentration (BAAC) appeared to decrease over the course of time in individuals with inactive *ALDH2*; and (5) BAAC profiles during the ethanol clamp procedure did not differ among the *ADH1B* genotypes for either the active or the inactive *ALDH2* carriers.

Using the same alcohol clamp paradigm, we evaluated changes in several plasma nitric oxide (NO) metabolites in blood associated with oxidative stress in response to low dose and well-controlled ethanol administration. After collecting peripheral blood from each subject before and at 30, 180, and 270 minutes after ethanol infusion, blood concentrations of nitrite and nitrate were measured by using high-performance liquid chromatography. These findings are summarized as follows: 1) despite the low dose, ethanol significantly reduced the level of blood NO (the sum of nitrite and nitrate) as early as 30 minutes after administration, and the blood NO level continued to be low at the completion of ethanol infusion; 2) there was little difference in the time profile of NO metabolite concentrations in the blood between subjects with active and inactive ALDH2. However, the concentration of NO metabolites for active ALDH2 subjects tended to return to baseline levels by 90 minutes after the end of ethanol infusion, whereas that tendency was not observed for the inactive ALDH2 subjects. These results suggest that low-dose continuous ethanol may increase the level of oxidative stress in the human body and that NO plays a minor role in the flushing response-related vasodilation.

We have also examined the influence of the genetic variants of the ADH and ALDH enzymes on alcohol-related medical disorders among Japanese with alcohol dependence. Our recently obtained findings suggest that the less-active allele of the ADH1B gene is associated with alcohol-induced persistent amnestic disorder (Matsushita et al., 2000) and cancer of the upper gastrointestinal tract (Yokoyama et al., 2001). Further, the inactive allele of the ALDH2 gene is associated with an increased risk for macrocytosis of the red blood cell (Yokoyama et al., 2003).

In other studies, we have examined the influence of ALDH2 genotypes on peripheral nerves and brain white matter in samples of alcoholics. In these studies, we compared the sensory nerve action potential amplitudes of the sural and median nerves and central sensory conduction time in somatosensory evoked potential (SEP) between Japanese alcoholics with active and inactive ALDH2. Our results revealed that compared with alcoholics with active ALDH2, 1) sensory nerve action potential amplitudes of median and sural nerves were lower in alcoholics with the *ALDH2*2* allele (Masaki et al., 2004); and 2) in alcoholics with the *ALDH2*2* allele, central sensory conduction time (P13/14 onset-N20 onset) corresponding to white matter conduction was prolonged (Mochizuki et al., 2004). Because the organs of alcoholics with inactive *ALDH2* are assumed to be exposed to high concentrations of acetaldehyde for long periods of time, these findings suggest that acetaldehyde may be involved in the pathogenesis of alcohol-related neurological disorders, including polyneuropathy and white matter damage.

RECENT FINDINGS RELATED TO THE GENETICS OF EXTERNALIZING BEHAVIOR PHENOTYPES AND ALCOHOL DEPENDENCE

Victor Hesselbrock, Danielle Dick, Tatiana Foroud, Michie Hesselbrock, John Nurnberger, Jr., Howard Edenberg, Marc Schuckit, Samuel Kuperman, Kathleen Bucholz, and John Kramer

Externalizing behaviors, including conduct disorder symptoms, aggression, and suicidal behavior are prevalent among individuals with psychiatric disorders, including alcohol dependence. A genome screen was performed in multiplex alcohol-dependent families ascertained as part of the Collaborative Study on the Genetics of Alcoholism (COGA) to identify chromosomal regions of interest related to three types of externalizing behaviors: suicidal behavior, aggression, and conduct disorder. Both the quantitative and qualitative phenotypes were based on information taken from the Semistructured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1995; Hesselbrock et al., 1999), a research diagnostic interview. Sibling pair analyses were used to conduct linkage analyses, using both qualitative and quantitative phenotypes of the externalizing behaviors under investigation. For the qualitative phenotype suicide attempts, chromosome 2 yielded evidence of linkage, whereas the quantitative suicidality index provided evidence of linkage on chromosome 3 and

chromosome 1. Significant evidence of linkage was found on chromosome 2 for the phenotype "suicide attempts," the same chromosomal region previously reported linked to alcohol dependence in this sample (Hesselbrock et al., 2004). This finding does not appear to be due solely to an association between suicide and alcohol dependence. There was no overlap in findings for theses two phenotypes of suicidal behavior. Evidence of linkage for the diagnostic phenotype of conduct disorder was found on chromosomes 2, 3, 12, and 19, whereas the quantitative conduct disorder symptoms index yielded findings on chromosomes 1 and 19 (Dick et al., 2004). The same region (about 130 to 134 cM) of chromosome 2 produced evidence of linkage for alcohol dependence in a previous study (conduct disorder and suicide attempts). No evidence of linkage was found for either of the quantitative traits of aggression or alcohol-related aggression.

ANTISOCIAL ALCOHOLISM AND RELATED PHENOTYPES: RECENT RESULTS FROM CLINICAL AND GENETIC STUDIES

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Alcoholism is frequently associated with a number of personality disorders and traits, especially antisocial personality, impulsivity, and aggression, which may also affect treatment outcome and prognosis. Research is very active in this field. Numerous studies point to serotonin (5-HT) as being a key neurotransmitter in this respect. Serotonergic neurotransmission appears to play a crucial role for both substance use and antisocial and impulsive behavior. A possible candidate gene for alcoholism, antisocial behavior, and related phenotypes is the 5-HT-1B receptor. 5-HT 1B receptor knockout mice show an increased alcohol intake, and 5-HT-1B agonists have been found to decrease aggression in the animal model. Findings of clinical and genetic studies as reviewed in this presentation have not been consistent in this field (Gorwood et al. 2002; Kranzler et al. 2002). Initially, an association and linkage of the 5-HT1B 861C allele with antisocial behaviors had been reported in two independent samples (Lappalainen et al. 1995). We studied the potential role of the 5-HT1B receptor polymorphism and antisocial behavior in alcohol dependence among 164 patients. The research instruments used to characterize and phenotype patients were in part adopted from the US COGA study. An association was found between a lower frequency of the 5-HT 1B 861C allele, antisocial personality traits, and conduct disorder in alcohol dependence. Adult antisocial personality was found more often in the male subjects. Based on the current data set, there are inconsistent but encouraging results supporting the role of the 5-HT 1B G 861 C polymorphism and antisocial behavior in alcohol dependence.

SUMMARY

The focus of this symposium was to provide new information on several alcohol-related phenotypes that are commonly found among clinical samples of alcohol-dependent persons. The first presentation by Higuchi et al. examined the influence of the genetic variants of the ADH and ALDH enzymes on alcohol-related medical disorders among Japanese with alcohol dependence. Through a series of studies of both affected and unaffected subjects, they were able to implicate the inactive form of ALDH in the development of several medical problems such polyneuropathy and gastric cancer and erythrocyte macrocytosis among chronic heavy drinkers. Hesselbrock et al. provided evidence that several alcohol-related psychiatric conditions characterized by externalizing behaviors may have some common underlying genetic determinants. Separate phenotypes based on suicidal behaviors, conduct disorder, and alcohol dependence each provided evidence of linkage to a similar region of chromosome 2 at 130 to 134 cM. Based on a sample of treated alcohol-dependent patients from Munich, Soyka conducted a candidate gene study of externalizing behavior/antisocial personality disorder focusing on genes from the serotonergic system. An association was found between a lower frequency of the 5-HT1B G861C allele and externalizing behavior and personality traits and alcohol dependence. Together, these three presentations focus on the importance of genetic susceptibility factors for developing alcohol dependence but also their importance in relation to the medical consequences of chronic drinking and the associated externalizing behaviors associated with alcohol dependence.

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