Biological and Behavioral Markers of Alcohol Sensitivity

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This article summarizes a symposium that was organized by Dr. Kim Fromme and presented at the 2003 annual meeting of the Research Society on Alcoholism in Ft. Lauderdale, Florida. The four presentations illustrate the emerging technologies and methods that are now being used to investigate the genetic basis of differential sensitivity to alcohol and their behavioral manifestations. Combining human genotyping with laboratory measures of behavior and subjective reports, these presentations represent state-of-the-art approaches to crossing the bridge from the Decade of the Brain to the Decade of Behavior. Dr. De Wit's paper describes her research on the neurobiological basis for individual differences in sensitivity to the stimulant and sedative effects of alcohol. Evidence suggests that activity of the dopaminergic and GABAergic neurotransmitters underlie these stimulant and sedative effects, respectively. Both Drs. Hutchison's and Corbin's papers describe their research on polymorphisms for the serotonin transporter (SLC6A4) as a determinant of the subjective effects of alcohol challenge. Dr. Hutchinson's and Ms. Ray's findings indicate that individuals with the short form of the SLC6A4 alleles (S) demonstrated a low level of response to alcohol, thus supporting previous research that the S allele may be associated with increased risk for alcohol dependence. In contrast, Dr. Corbin did not find a reliable association between the SLC6A4 genotype and subjective response to alcohol. Mr. Cook's and Dr. Wall's paper adds another dimension to this article by presenting research on both the aldehyde dehydrogenase (ALDH2) and alcohol dehydrogenase (ADH2) genetic variants and their association with the alcohol-related flushing response that is prevalent in Asian populations. Dr. David Goldman provides concluding remarks.

Key Words: Alcohol Sensitivity, Serotonin, GABA, ALDH2, ADH2.

THERE ARE LARGE individual differences in response to alcohol, and recent evidence indicates that these differences confer relative risk for alcohol dependence (e.g., Schuckit et al., 1999). A lower response to certain effects of alcohol (e.g., feelings of high) and a greater response to other effects (e.g., facial flushing) both have been shown to influence alcohol use patterns and the development of alcohol-related problems. All of such physiological, psychological, and behavioral effects of alcohol are determined by activity of the central nervous system (CNS) and related neurotransmitter activity.

More than 100 neurochemicals have been identified, and many have been associated with alcohol use and dependence (for review, see Fromme and D'Amico, 1999). γ-Aminobutyric acid (GABA) has long been the focus of research on the sedative or anxiolytic effects of alcohol, whereas dopamine has been explored intensely as the basis for the rewarding effects of drinking. Serotonin seems to have widespread effects throughout the CNS, for example with acute alcohol intake being associated with higher levels of 5-HT (LeMarquand et al., 1994). Aldehyde dehydrogenase (ALDH) has been explored as a potential protective mechanism against the development of alcoholism (Wall and Ehlers, 1995). A deficiency in one of four isoenzymes of ALDH, the ALDH2, has been associated with alcohol-induced flushing and alcohol sensitivity, possibly contributing to lower levels of alcoholism among those with this genetic polymorphism.

The presentations in this symposium illustrate the potential importance of genetic variation as one determinant of individual differences in response to alcohol but also the complexities in attempts to understand how neurotransmitter activity relates to the development of alcohol-related problems and dependence.

INDIVIDUAL DIFFERENCES IN SUBJECTIVE RESPONSES TO ALCOHOL IN HUMANS

Harriet de Wit

Most people try alcohol at least once in their lives, and most continue to consume it at moderate levels, without problems, throughout their lives. However, for some people, use of the drug escalates to levels that interfere with normal life function, sometimes with devastating consequences. Why do some individuals continue to use alcohol at moderate levels whereas others develop severe problems of alcohol abuse and dependence? Researchers have identified many factors that influence the development of alcohol abuse and dependence, including sociocultural factors, such as drug availability, legality, and social mores, and biological factors, such as genetics, tolerance, and body composition (Tarter, 2002).

Our laboratory has investigated individual differences in the quality and magnitude of the drug's direct, mood-altering effects as possible determinants of repeated alcohol use. Both casual observations of drinkers and controlled laboratory studies show that alcohol produces markedly disparate effects in different individuals (de Wit et al., 1987, 1989). In some people, it produces feelings of stimulation and well-being, whereas in others, it produces depression, sedation, and even in some people, aggression. The laboratory-based studies confirm these differences while controlling for expectancies (using placebo beverages), alcohol dose, and drinker characteristics such as age, sex, drinking history, race, psychiatric symptoms, and socioeconomic status. Even when all of these factors are controlled, there remain stable qualitative differences in the subjective, or mood-altering, effects experienced by different individuals (de Wit et al., 1987, 1989; Holdstock and de Wit, 1999). Specifically, some individuals experience primarily stimulant-like effects from alcohol, including feelings of increased energy, sociability, and euphoria, whereas others report primarily sedative-like effects, including feelings of fatigue and low energy. Although the stimulant effects are especially common among heavier drinkers (Holdstock et al., 2000; King et al., 2002), the individual differences are observed even among light social drinkers, who consume as little as five drinks per week (de Wit et al., 1987). We have shown that these differences in subjective effects of alcohol are also related to consumption. Drinkers who experience primarily stimulant-like effects from alcohol report liking the effects, and when they are given a choice between an alcohol beverage and a placebo, they choose the alcohol. In contrast, people who experience sedative effects generally do not like the effects, and they choose the placebo. This link between the qualitative subjective effects of alcohol and its reinforcing effects (as measured by preference tests) led us to examine more closely factors that may determine the individual differences in subjective responses, including sensitivity of neurotransmitter receptor systems and endocrine systems.

CNS Receptor Sensitivity

One potential source of variation in subjective effects of alcohol is differences in the sensitivity of the receptor systems, where alcohol has its effects in the brain. Alcohol is known to act on multiple neurotransmitter systems, including dopamine (DA), noradrenaline, serotonin, opioid, acetylcholine, GABA, and glutamate (Tabakoff et al., 1996). Of these systems, the DA system is associated mainly with stimulant-like effects of drugs and the GABA system with sedative-like effects. Thus, we reasoned that individuals with relatively more sensitive DA receptor systems may be more sensitive to the stimulant-like actions of ethanol, whereas individuals with more sensitive GABA systems may be more likely to experience the sedative-like effects of alcohol (Holdstock and de Wit, 1999, 2001). To assess sensitivity of these systems, we administered drugs with known actions at these receptor systems: d-amphetamine to assess the DA system and triazolam to assess the GABA system. In the first study, we examined individual differences in the stimulant effects of alcohol in relation to amphetamine, a drug that acts via DA systems.

There is considerable preclinical evidence that the stimulant-like behavioral and physiological effects of ethanol are mediated through DAergic systems (Cohen et al., 1997). Ethanol increases the firing rate of DA neurons and stimulates DA turnover, metabolism, and release (Imperato and Di Chiara, 1986). Behaviorally, ethanol increases locomotor activity in animals, which is blocked by treatments that reduce DA function (Cohen et al., 1997). DA antagonists injected directly into the nucleus accumbens block alcohol drinking in rats, whereas DA agonists into these areas prolong drinking in rats (Samson et al., 1992). Despite these studies with animals, relatively few studies have examined the role of DA in ethanol responses in humans. We (Holdstock and de Wit, 2001) studied individual differences in responses to the DA agonist amphetamine (AMP) and ethanol in 27 male and female social drinkers. Subjects participated in four sessions in which they received beverages or capsules containing ethanol (0.8 g/kg), AMP (10 or 20 mg), or placebo and completed self-report mood questionnaires and objective tests at regular intervals after taking the drug. Consistent with our earlier findings (de Wit et al., 1987, 1989), approximately half of subjects reported primarily stimulant-like effects; the other half reported primarily sedative-like effects. To examine the relationship between the two drugs, we examined the correlation in peak effects between the ethanol and AMP (20 mg). Consistent with our hypothesis, responses to ethanol were positively correlated with responses to AMP (20 mg) on a measure of stimulant-like subjective effects ($r = 0.41$, $p < 0.05$). This correlation was even higher among the subjects who reported primarily stimulant-like effects from ethanol (0.64, $p < 0.05$). This positive correlation between stimulant responses to the two

drugs suggests that their stimulant effects may be mediated through similar mechanisms.

Further evidence for a role of DA in the stimulant-like and reinforcing effects of ethanol comes from another recent study (Enggasser and de Wit, 2001) in which we examined the effect of a DA antagonist, haloperidol, on the subjective stimulant-like effects of ethanol. Healthy volunteers ($n = 17$) were pretreated with haloperidol (3 mg) or placebo before consuming ethanol (0.75 g/kg) or placebo. They completed self-report measures after these doses, and then, after the third beverage, they were permitted to consume up to five additional unit doses of the beverage that they had ingested. We found that haloperidol reduced ethanol beverage choice without altering placebo choices. Notably, haloperidol reduced the stimulant-like and euphorigenic effects of ethanol in subjects who experienced stimulant effects ($n = 8$), whereas it had no effect in subjects who did not experience stimulation ($n = 9$). These findings support the idea that individual differences in the stimulant effects of ethanol are related to DA function.

We have also investigated individual differences in responses to ethanol in relation to GABA function, by administering both ethanol and triazolam to the same subjects (Holdstock and de Wit, 1999). There is considerable evidence that ethanol produces its sedative-like effects through actions on the GABA receptor complex (Grant and Lovinger, 1995). In our study, healthy volunteers ($n = 27$) received doses of ethanol (0, 0.2, 0.4, and 0.8 g/kg) or triazolam (0, 0.125, 0.25, and 0.5 mg) and completed self-report questionnaires and behavioral tests. As in our previous studies, approximately half of the subjects experienced sedative effects from alcohol. Among subjects who experienced sedative effects, there was a positive correlation between level of sedation produced by triazolam and ethanol ($r = 0.66$, $p < 0.01$). This correlation provided support for the idea that sensitivity of the GABA receptor system plays a role in the intersubject variability in sedative-like effects of ethanol.

Stress Reactivity

Abnormalities in neuroendocrine systems and stress reactivity have been linked to susceptibility to alcoholism and drug abuse (Dawes et al., 1999). For example, Marinelli and Piazza (2002) found that locomotor activity in an open-field test in rats is a predictor of drug self-administration. Rats that exhibit more locomotor activity more readily self-administer stimulant and opiate drugs. These "high responder" rats also exhibit a larger increase in corticosterone after acute restraint stress. It is not known whether there is a human analog of these high- and low-responder rats. However, we recently analyzed an existing data set (de Wit et al., 2003) to examine the relationship between stress reactivity and alcohol consumption in humans. On one session in this study, subjects performed a speech and arithmetic task (Trier Social Stress Test; Kirschbaum et al., 1993), which increases cortisol levels in most subjects, and on a separate session they were permitted to consume up to six unit doses of alcohol. On the basis of the findings with laboratory animals, we hypothesized that the subjects who exhibited the greatest increase in cortisol after the stress test would consume the most alcohol in the consumption session. Consistent with the hypothesis, we found that the correlation between peak cortisol level after stress (in one session) and percentage of available alcohol consumed (in a separate session) was $r = 0.4$, consistent with the animal findings. Although this finding is highly preliminary, it suggests that in humans, as in animals, some of the variability in alcohol consumption may be related to reactivity of the hypothalamic-pituitary-adrenal axis.

The studies described above suggest that acute challenge studies offer a valuable approach to studying vulnerability to alcohol or other drug use. We have found repeatedly that social drinkers vary widely in their subjective responses to ethanol, in ways that are related to their tendency to consume alcohol. Individuals who experience stimulant-like effects from ethanol tend to like it and choose it over a placebo, whereas individuals who experience sedative effects tend to dislike ethanol. Studies such as those described above may begin to elucidate the neurochemical mechanisms that are associated with the stimulant-like and positive reinforcing effects of alcohol. The variability in acute responses to alcohol typically observed in nonproblem social drinkers provides a rich source of information regarding possible risk factors for developing problems with excessive use; individuals with the stimulant-like pattern of response to alcohol, who are exposed to the appropriate permissive psychosocial conditions, may proceed along a predictable trajectory to problematic levels of use.

SENSITIVITY TO ALCOHOL IN HUMANS: INFLUENCE OF THE 5-HT TRANSPORTER POLYMORPHISM

Kent E. Hutchison and Lara Ray

Alcoholism is a complex disorder with a strong genetic component that may account for approximately half of the variability in risk (Heath and Phil, 1995). Over the past several years, considerable research efforts have attempted to identify genetic markers for alcohol abuse and dependence. One polymorphism in the 5' promoter region (5'HT-TLPR) of the serotonin transporter gene (locus ID SLC6A4) is of particular interest as studies have demonstrated its functional relationship to the availability of serotonin transporter both in vitro (Heils et al., 1996) and in vivo (Heinz et al., 2000). This 44-bp deletion/insertion polymorphism results in two common alleles, the 528-bp "long" allele (L) and the 448-bp "short" allele (S). The S allele has been shown to decrease transcription and decrease 5-HT reuptake (Heils et al., 1996). In addition, several studies have found an association between the S allele and the alcohol dependence diagnosis in both case and control designs (Hammoumi et al., 1999; Sander et al., 1998; Thompson et al., 2000) and family-based studies (Lichter-

mann et al., 2000). In contrast, several investigations have not been able to replicate the relationship between the S allele of the 5'HTTLPR polymorphism and alcohol dependence (e.g., Edenberg et al., 1998).

In a study by Schuckit et al. (1999), individuals with the S allele showed higher sensitivity to the effects of alcohol compared with individuals with the L allele. These results contradict the findings of Turker et al. (1998), which suggested a positive association between high alcohol tolerance and the S variant of the 5'HTTLPR polymorphism. Thus, not only are reports of an association between this polymorphism and alcohol dependence contradictory, but also reports on the relation between this polymorphism and alcohol sensitivity as an intermediate phenotype are inconclusive and warrant further investigation. The current study was designed to test the association between the 5'HTTLPR polymorphism and the intermediate phenotype of alcohol sensitivity.

The research was approved by the University of Colorado Human Research Committee. A total of 113 participants (49 women) between the ages of 21 and 35 were recruited from the greater University of Colorado community. Participants were included in the research when they demonstrated moderate to heavy drinking [i.e., drank more than five drinks (four for women) at least twice per week], did not report any use of psychotropic medications or use of illicit drugs with the exception of marijuana, did not report any hearing loss, had never been treated or were not currently seeking treatment for an alcohol-related problem, and were negative on a pregnancy test (women only). The sample was primarily of Caucasian ancestry (96%), although individuals also noted some African ancestry (2%), Asian ancestry (6%), and Native American ancestry (4%). The mean age of the sample was 22.6 (SD = 2.1). During the 30 days before participation in the study, the mean number of drinks per drinking day was 4.8 (SD = 2.3) and the mean number of days with a least one drink was 13.4 (SD = 5.6). Participants received $50 for completing the study. These data were collected as one component of a larger project on reactions to alcohol.

After arriving at the laboratory, participants completed a baseline packet of questionnaires and then were allowed to relax before completing the alcohol challenge. The alcohol challenge consisted of three standardized alcoholic drinks (a high-alcohol-content beer). The amount of alcohol [target breath alcohol level of 0.06) was calculated using a nomogram that factors in the influence of height, weight, and sex with each containing 0.15 g/kg doses of alcohol for men (0.11 g/kg for women). Participants consumed each of the three doses of alcohol approximately 20 min apart. The mean breath alcohol level after each dose of alcohol, as estimated by an AlcoSensor IV, was 0.023, 0.041, and 0.056, respectively. After consuming each beverage, participants completed the assessments of subjective high including a single-item scale as well as a 13-item scale assessing subjective levels of intoxication adapted from the one described by Schuckit and Gold (1988).

Genomic DNA was collected at baseline and isolated from buccal cells using published procedures (see Freeman et al., 1997). The 5'HTTLPR polymorphism of the SLC6A4 was assayed using a modification of published procedures (see Lesch et al., 1996). The primer sequences were forward 5'-GGCGTTGCCGCTCTGAATGC-3' and reverse 5'-GAGGGACTGAGCTGGACAACCAC-3', yielding products of 484 bp (S allele) or 528 bp (L allele).

Consistent with previous reports, analyses indicated an increase in subjective high across drinks. In addition, analyses suggested that subjective high increased most rapidly among individuals homozygous for the L allele followed by the heterozygous individuals and the individuals homozygous for the S allele. Analyses of the 13-item scale were consistent with the single-item scale.

Conclusions

Findings indicate that the 5'HTTLPR polymorphism of the SLC6A4 is associated with subjective high during the consumption of alcohol such that individuals with the S allele experience less subjective high while drinking. These results are consistent with a previous report that suggested that individuals with the S allele demonstrated less sensitivity to alcohol (Turker et al., 1998) but inconsistent with another investigation in which individuals with the L allele experienced an attenuated response to alcohol (Schuckit et al., 1999).

Given the conflicting results across studies, it is difficult to reach firm conclusions regarding the influence of this polymorphism on acute responses to alcohol or the development of alcohol dependence. Conflicting results may be due to differences in methods across studies, and replication within the context of a specific experimental paradigm will be necessary to increase confidence in previous findings. Population stratification is another often-cited reason for the lack of consistent results in candidate gene studies. However, population stratification is generally less of a threat than other factors, including unidentified environmental or genetic factors, that may influence these same intermediate phenotypes (e.g., Hutchison et al., 2004), and in any case is an unlikely source of bias in the present study given the relatively homogeneous ancestry of the sample. An important implication of the present findings and previous studies noting an association between this polymorphism and acute responses to alcohol is that future studies will need to control for this polymorphism when analyzing the effect of other candidate polymorphisms on similar phenotypes (e.g., polymorphisms in the mu opiate receptor gene OPRM1).

ASSOCIATION AMONG A FUNCTIONAL SEROTONIN TRANSPORTER POLYMORPHISM, SUBJECTIVE ALCOHOL RESPONSES, AND AD LIBITUM CONSUMPTION

William R. Corbin

It is well established that genetic factors contribute substantially to the risk for alcohol abuse and dependence with estimates in the range of 40 to 60% (Schuckit, 2000). What

is less clear are the mechanisms through which genetic factors influence drinking outcomes. Because of the complex nature of alcohol use disorders, researchers have begun to identify endophenotypes that are more easily defined and quantified. For example, genetic risk may be associated with differences in personality, event-related potentials, and subjective alcohol effects, each of which are known to be associated with alcohol-related problems. With respect to subjective alcohol effects, considerable evidence exists for genetic influences, although the nature of these effects are a continued source of debate.

Using both subjective and objective measures, Schuckit and colleagues have shown that young men with a family history of alcoholism demonstrate a less intense response to alcohol relative to those without a family history (Schuckit, 1988; Schuckit and Gold, 1988; Schuckit et al., 1987). In addition, a low subjective response in early adulthood is associated with risk for alcoholism 15 years later (Schuckit, 1998). A meta-analysis supported the association between family history of alcoholism and a low subjective response to alcohol (Pollock, 1992), and data from the Collaborative Study on the Genetics of Alcoholism showed a similar pattern of results (Schuckit et al., 1996).

Presumably, a low subjective response to alcohol leads to increased risk for alcoholism via increased consumption. Individuals who do not feel the effects of alcohol as strongly may drink more to obtain the same effects that others experience at a lower blood alcohol level (BAL). Although it is intuitively appealing, contradictory data call this theoretical model into question. Researchers from a variety of laboratories have failed to support an association between family history of alcoholism and a low subjective response, instead finding that these individuals have comparable or stronger responses to alcohol as measured by self-report (Newlin and Thomson, 1991), heart rate (Conrod et al., 1997; de Wit and McCracken, 1990), and hormonal levels (Gianoulakis et al., 1996).

In a review of the literature, Newlin and Thomson (1990) attempted to incorporate the disparate findings, concluding that high-risk individuals are less sensitive to the sedating effects of alcohol experienced on the descending limb of the blood alcohol curve but more sensitive to the stimulating effects on the ascending limb. A similar but slightly different hypothesis is that high-risk individuals have a stronger initial response to alcohol but more quickly develop acute tolerance to alcohol effects. An elegant experiment in which BAL was held constant provided strong support for this hypothesis. Morzorati et al. (2002) found that individuals with a positive family history experienced stronger initial responses to alcohol but developed acute tolerance to these effects more quickly. It remains to be seen whether reduced sedative effects in high-risk individuals reflect differential sensitivity to sedation or more rapid acute tolerance to alcohol effects more generally.

The current study evaluated the association between subjective alcohol effects (30–60 min after consumption) and ad libitum alcohol consumption in a simulated bar laboratory. This design provided an opportunity to test the hypothesized association between low subjective responses and drinking behavior while also including multiple assessments of subjective effects to assess potential changes in this relationship across time. In addition, genetic samples were collected and PCR analyses were conducted to characterize participants with respect to genotype for the SCL6A4 serotonin transporter (SERT) polymorphism. Schuckit et al. (1999) found that the long allele of the SERT gene was associated with a low subjective response to alcohol and an increased risk for alcoholism in a subgroup of their longitudinal sample.

Participants in the current study were recruited as part of a prevention program in which pre- and posttest assessments included alcohol administration. Analyses were based on data collected at the preintervention assessment. Genetic samples were collected from a subset of the original sample approximately 2 years after the completion of the prevention study. Valid PCR data for the SERT genotype were available for 83 of the 90 participants who completed the follow-up assessment. Within this subsample, the mean age was 21.6 (SD = 3.1), 68% were male, and 65% were European-American. Other groups prominently represented were Latina (19%) and Asian-American (10%).

After being dosed to a target BAL of 0.06 mg%, participants' BAL was assessed and they rated their level of subjective "intoxication" on a 100-mm visual analog scale. Several unrelated cognitive tasks were completed, and then BAL and subjective experiences were reassessed. A standardized social stressor was introduced whereby participants were told that they would have 20 min to prepare for a 5-min self-disclosing speech. During the speech preparation time, participants were given access to alcoholic and nonalcoholic beverages and project staff monitored their intake to ensure that no participant exceeded a BAL of 0.12 mg%.

Multiple regression analysis was used to assess subjective intoxication as a predictor of ad libitum consumption, controlling for the effects of sex, typical weekly alcohol consumption, and BAL. Separate analyses were conducted for the 30- and 60-min assessments. Preliminary results indicated that low subjective intoxication was associated with greater alcohol consumption at both time points. Additional regression analyses were conducted to assess the relation between SERT genotype, ad libitum consumption, and subjective intoxication. SERT genotype was unrelated to ad libitum consumption or subjective intoxication at either time point.

Consistent with the theoretical model, a low subjective response to alcohol was associated with increased ad libitum alcohol consumption. This association was found at both 30 and 60 min after alcohol administration. These findings add to the growing body of research suggesting that subjective responses are an important marker of risk for alcohol use disorders. Contrary to study hypotheses, the

long allele of the SERT gene was not associated with ad libitum consumption or low subjective response to alcohol. Although this represents a failure to replicate the results of the Schuckit et al. (1999) study, the differences may have been due to the timing of assessments. It is possible that the lower subjective responses in the Schuckit et al. (1999) study reflected acute tolerance rather than an overall lower level of response.

The lack of association between SERT and ad libitum consumption suggests that heavier drinking may not be the mechanism through which SERT genotype leads to increased risk for alcoholism. Nonetheless, the long allele of the SERT gene may lead to increased risk for alcoholism via other mechanisms. For example, Heinz and Goldman (2000) suggested that individuals with the long allele may be more susceptible to the long-term toxic effects of alcohol.

In summary, this study confirmed that a low subjective response to alcohol is a risk factor for heavy drinking as assessed in a laboratory setting. This fits nicely with the theoretical model suggesting that individuals with a low subjective response to alcohol will drink more to achieve the same effects that others can achieve at a lower dose. The results of the genetic analyses neither confirmed nor disconfirmed an association between SERT genotype and alcoholism but suggest that any association that does exist is not likely mediated by level of consumption.

ASSOCIATIONS OF *ALDH2* AND *ADH1B2* GENOTYPES WITH RESPONSE TO ALCOHOL IN ASIAN AMERICANS

Travis A.R. Cook and Tamara L. Wall

The two genes with the strongest known associations with alcohol dependence encode isoenzymes involved in alcohol metabolism. Genes for alcohol dehydrogenase (*ADH1B* located on chromosome 4) and aldehyde dehydrogenase (*ALDH2* on chromosome 12) exhibit functional polymorphisms that have been related to lower rates of alcohol dependence. The *ALDH2*2* allele, which is prevalent in Asian populations but extremely rare in non-Asians, has the strongest relationship. Studies conducted among Chinese, Taiwanese, Japanese, Koreans, and Thais have found that individuals who are homozygous for *ALDH2*2* have nearly zero risk, whereas heterozygotes are approximately one third as likely to be alcohol dependent compared with those without this allele (Assangkornchai et al., 2003; Chen et al., 1999; Chen et al., 1996; Higuchi et al., 1994; Lee et al., 2001; Muramatsu et al., 1995; Shen et al., 1996; Thomasson et al., 1991).

The *ADH1B*2* allele has also been associated with lower rates of alcohol dependence. This relationship has been found after controlling for the effect of *ALDH2*2* in Asian subgroups (Chambers et al., 2002; Chen et al., 1999; Chen et al., 1996; Higuchi et al., 1994; Muramatsu et al., 1995; Shen et al., 1996; Thomasson et al., 1991), and has also been found in Caucasian subgroups (Borras et al., 2000;

Whitfield et al., 1998). A recent meta-analysis indicated that the risk for alcohol dependence is between one third to one half among individuals with *ADH1B*2* alleles compared with those without this allele (Whitfield, 2002).

The process by which these two genetic polymorphisms ultimately give rise to differences in rates of alcohol dependence is hypothesized to be via elevations in acetaldehyde, an intermediary metabolite produced during the conversion of alcohol to acetate. On the basis of their kinetic properties, *ALDH2*2* theoretically should lead to slower removal of acetaldehyde than *ALDH2*1*, whereas *ADH1B*2* should lead to faster production of acetaldehyde than *ADH1B*1* (Bosron and Li, 1986). It is also hypothesized that individuals with these alleles should demonstrate increased sensitivity to the acute effects of alcohol. On the basis of previous association studies of these genes with risk for alcohol dependence, we hypothesized that individuals who were homozygous for *ALDH2*2* would have the most intense reactions to alcohol and that *ALDH2*2* heterozygotes would have more intense reactions than those without this allele. We further hypothesized that within each of the *ALDH2* genotype groups, those who were homozygous for *ADH1B*2* would have the most intense reactions to alcohol and that *ADH1B*2* heterozygotes would have more intense reactions than those without this allele.

To test these hypotheses, we evaluated physiological and subjective reactions before and at 30-min intervals for 150 min after ingestion of placebo and alcohol beverages among 101 (14 female) young adults (average age 21.8 years, range 21–26 years) of Chinese, Japanese, or Korean heritage who were genotyped at the *ALDH2* and *ADH1B* loci. *ALDH2* genotyping indicated that 61 participants were 1*1, 37 were 1*2, and 3 were 2*2. *ADH1B* genotyping indicated that 7 participants were 1*1, 39 were 1*2, and 55 were 2*2. Procedures to control for possible confounding variables that might influence level of response to alcohol included the following: (1) all participants lacked a history of alcoholism among first-degree relatives; (2) alcohol was dosed on the basis of body weight or estimated body water to achieve a target BAL of approximately 0.08; (3) phase of the menstrual cycle was controlled for women; and (4) recent (past 6 month) drinking history was restricted to <50 drinks per month for men and <25 drinks per month for women. During the 6 months before testing, the 101 participants had consumed alcohol on average 4.0 (SD = 2.9) times per month and drank on average 2.4 (SD = 1.4) standard drinks per occasion. Experimental variables were also controlled, such as time of day (beverage ingestion occurred at 9:00 AM) and the amount of time for beverage consumption (7–15 min). Response to alcohol and placebo were assessed using BAL, observed facial flushing, pulse rate, cortisol, and subjective feelings of intoxication using the Subjective High Assessment Scale.

As we previously reported, results suggest distinct phenotypic differences in response to alcohol associated with the three *ALDH2* genotypes (Luczak et al., 2002; Wall et

al., 1992). In both men and women, *ALDH2*2* heterozygotes subjectively reported more intense but not less pleasurable reactions to alcohol than those with *ALDH2*1/2*1* genotype despite virtually identical self-reports after placebo and equivalent BALs after alcohol. The overall more intense subjective response to alcohol experienced by *ALDH2*2* heterozygotes was also observed using objective measures of intoxication (facial flushing, pulse rate, and cortisol levels). In addition, each of the three *ALDH2*2* homozygotes experienced even more intense subjective and objective reactions to alcohol than heterozygotes. All three became tachycardic, hypotensive, nauseated, and emetic after ingestion of the alcohol beverage. The *ADH1B*2* allele was also associated with more intense reactions to alcohol, although these effects were not as large as the effects of *ALDH2*2* and were only significant in *ALDH2*2* heterozygotes. Among these participants, *ADH1B*2* homozygotes were more likely to become emetic and subjectively reported feeling less "Great overall" on the SHAS following alcohol than *ADH1B*2* heterozygotes.

The results from this study suggest that Asians who are homozygous for *ALDH2*2* have severe and predominantly negative reactions to a moderate dose of alcohol and that *ALDH2*2* heterozygotes have overall more intense objective and subjective reactions than individuals without this allele. The presence of an *ADH1B*2* allele was also associated with more intense reactions to alcohol but only among those with *ALDH2*1/*2* genotype. The additive effects of one *ALDH2*2* and one *ADH1B*2* allele represented predominantly negative responses (e.g., increased emesis and feeling less "Great overall") and were most similar to the responses of *ALDH2*2* homozygotes. These findings are consistent with the hypothesis that increased sensitivity to alcohol is the mechanism by which these alleles combine to protect against alcohol dependence.

It is also important to consider these results within the context of several limitations. Because only moderate drinkers were included in this study, results may not generalize to infrequent or heavy drinkers. Furthermore, individuals with the most intense responses to alcohol were probably unlikely to volunteer to participate in this study. Finally, small sample sizes for ethnic subgroups precluded the examination of ethnicity effects.

DISCUSSION AND CONCLUSIONS

David Goldman

Alcohol sensitivity and level of response to alcohol have emerged as one of the most promising and active research targets for research on alcoholism cause. These four papers reveal progress in understanding the relationship between alcohol consumption and these intermediate phenotypes and in the first steps to identify the role of genetic variation. It is well understood that alcoholism is a complex, genetically influenced disease, with a heritability of approximately 50% and both substance-specific and substance-nonspecific

causality (Goldman and Bergen, 1998). Two aspects of the etiologic redefinition of alcoholism, a clinical entity, are the identification of intermediate (mediating) phenotypes and gene variation responsible for inheritance. As discussed by de Wit, in humans, alcohol response is heritable, and low alcohol response predicts higher levels of alcohol consumption and future problems with alcohol, as first shown in the groundbreaking studies of Schuckit at UCSD.

Variation in which systems determines variation in alcohol response? Pharmacogenetic variation can be broadly divided into pharmacokinetic variation and pharmacodynamic variation. Pharmacokinetic variation encompasses drug absorption, distribution, and metabolism, and it is easily understood that pharmacokinetic processes can contribute a substance-specific component to alcoholism vulnerability. For example, the ADH1B His 47Arg and ALDH2 Glu487Lys polymorphisms researched by Cook and Wall alter alcohol metabolism and directly lead to alcohol-induced flushing in East Asians. Alcohol flushing is a salient and important alcohol response, conferring substantial protection against alcoholism. However, it is predominantly an aversive syndrome extraneous to alcohol's actions on the brain: there is some evidence that acetaldehyde in the brain may actually be reinforcing. However, in both mice and humans, variation in alcohol response is primarily pharmacodynamic in origin, involving differences in neuronal response. Therefore, the question may be more precisely restated as, "What is the transceiver that is altered in some people?" Other work presented here offers some clues.

The other main category of pharmacogenetic variation is the pharmacodynamic response of cell and tissue. Genes that act on pharmacodynamics can also be specific or general in their effect. One important mechanism of alcohol addiction that may provide a shared link between alcoholism and other behaviors is activation of the brain stress axis (reviewed in Barr and Goldman, 2002). de Wit presented evidence from an acute alcohol administration study that hyperresponsivity of the stress axis predicts increased alcohol intake in humans. As she observed, elevations of cortisol and other stress-axis measures have repeatedly been observed in alcoholics. Thus, elevated stress response could mediate both initial response to alcohol and the progression to addiction and would of course influence vulnerability to other problems such as depression and anxiety, which have been repeatedly tied to both alcoholism and stress.

$GABA_A$ receptors are among the ligand gated ion channels most strongly implicated by cellular electrophysiology studies in alcohol effects on the brain (other strongly implicated and perhaps by coincidence functionally related receptors are the N-methyl-D-aspartate (NMDA) receptor and nicotinic receptors). de Wit's work in humans extends studies in mice implicating $GABA_A$ receptors as a primary target for genetic variation in alcohol response. It is interesting that specific $GABA_{A\alpha 6}$ subunit amino acid substitutions have previously been linked to alcohol and benzodi-

azepine response in the rat (Korpi et al., 1993) and in my laboratory at the National Institutes of Health, in the human (Iwata et al., 1999; Schuckit et al., 1999). Although the same human GABA$_A$ polymorphism has preliminarily been implicated in both alcohol and benzodiazepine response, genetic variation in response to these two drugs is likely to have both shared (common genes) and unshared origins, as indicated by studies finding only partial correlations in responses of both humans and rodent strains to these drugs.

Hutchinson, Ray, and Corbin have investigated the role of the SERT promoter polymorphism in alcohol response, with results that vary from each other and from our previous study on SERT and alcohol response (Schuckit et al., 1999). SERT is a functional polymorphism that could alter alcohol response either by altering stress response or at the direct level of serotonin effect, because alcohol has been shown to release serotonin and may modulate the 5HT3 receptor. Individuals with the S allele have repeatedly been shown to be more anxious and dysphoric, and within the past 2 years, the S allele was shown to predict enhanced amygdala response to a cognitive fear challenge (Hariri et al., 2002), and the effect of the S allele to increase risk of depression was tied to stress exposure (Caspi et al., 2003). Thus, it is not surprising if the SERT polymorphism alters alcohol response, as we reported (Schuckit et al., 1999). However, associations of SERT to alcohol response could be highly context dependent. The genetic sample of Schuckit and colleagues was relatively small, and our finding was preliminary. By expanding the number of individuals studied as in the new studies reported here, by refining or varying the response paradigm, and by homing in on differences between high and low responders, we may be able to isolate a role for SERT as one modifier of alcohol response.

A final important clue to the molecular origins of variation in individual vulnerability to alcoholism is that alcohol response varies between individuals not only quantitatively but also qualitatively. As de Wit discussed, some individuals experience more stimulant-like effects than others. Intriguing is that alcoholics are more likely to experience ketamine, an NMDA, antagonist, as similar to alcohol, but they perceive the effect as descending-limb effects rather than ascending-limb effects (Krystal et al., 1998). Alcohol also seems to act at least in part by blocking NMDA receptors. As the ability of genetic variation and environmental modulators to affect the function of NMDA, GABA$_A$, and other receptors is better understood, the inherited and induced vulnerability to alcoholism and the specific role of alcohol response will be clarified.

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