

Functional Relevance of Human *ADH* Polymorphism

C. J. Peter Eriksson, Tatsushige Fukunaga, Taisto Sarkola, Wei J. Chen, C. C. Chen, J. M. Ju, Andrew T. A. Cheng, Hidetaka Yamamoto, Kathrin Kohlenberg-Müller, Mitsuru Kimura, Masanobu Murayama, Sachio Matsushita, Haruo Kashima, Susumu Higuchi, Lucinda Carr, D. Viljoen, L. Brooke, T. Stewart, T. Foroud, J. Su, Ting-Kai Li, and John B. Whitfield

This article represents the proceedings of a symposium at the 2000 ISBRA Meeting in Yokohama, Japan. The chairs were C. J. Peter Eriksson and Tatsushige Fukunaga. The presentations were (1) 4-Methylpyrazole as a tool in the investigation of the role of ADH in the actions of alcohol in humans, by Taisto Sarkola and C. J. Peter Eriksson; (2) *ADH2* polymorphism and flushing in Asian populations, by Wei J. Chen, C. C. Chen, J. M. Ju, and Andrew T. A. Cheng; (3) Role of *ADH3* genotypes in the acute effects of alcohol in a Finnish population, by Hidetaka Yamamoto, Kathrin Kohlenberg-Müller, and C. J. Peter Eriksson; (4) Clinical characteristics and disease course of alcoholics with different *ADH2* genotypes, by Mitsuru Kimura, Masanobu Murayama, Sachio Matsushita, Haruo Kashima, and Susumu Higuchi; (5) *ADH2* polymorphism, alcohol drinking, and birth defects, by Lucinda Carr, D. Viljoen, L. Brooke, T. Stewart, T. Foroud, J. Su, and Ting-Kai Li; and (6) *ADH* genotypes and alcohol use in Europeans, by John B. Whitfield.

Key Words: ADH2, ADH3, Polymorphism, Alcohol Drinking, Flushing, Birth Defects.

THE BULK OF human alcohol metabolism takes place in the liver, where alcohol is first oxidized by alcohol dehydrogenase (ADH) to acetaldehyde, which is then oxidized by aldehyde dehydrogenase (ALDH) to acetate. All alcohol effects primarily can be derived from alcohol per se and/or its metabolism, including redox changes and the production of acetaldehyde and acetate. The rate of alcohol oxidation is the crucial factor that determines the metabolic consequences during alcohol intoxication. The hepatic NADH reoxidation together with the functional ADH and ALDH activities regulate the steady-state alcohol oxidation rate. Thus, any genetic polymorphism that affects

functional ADH and ALDH activities may be relevant for the biological actions of alcohol.

The first gene shown to affect the biological actions of alcohol was *ALDH2*. Deficiency of the ALDH2 enzyme, common in Asian populations, follows the pattern of a classical inborn error of metabolism, with dominant inheritance and high penetrance of the phenotype, which involves facial flushing due to blood vessel vasodilation, tachycardia, nausea, and headache in response to alcohol intake. These reactions, caused by inhibited acetaldehyde metabolism and subsequently elevated acetaldehyde levels, make alcohol drinking less pleasant and protect the individual from high consumption and alcoholism. Virtually no case of alcoholism has been reported in those individuals (about 5% to 10% of the entire Asian population) who are homozygous for the genotype (*ALDH2**2/*2). The heterozygous individuals with one *2-allele (30% to 40% of the entire Asian population) can drink but develop a number of side effects that include flushing. Within these individuals, the average alcohol consumption is less than in the *1/*1 population, but still alcoholism may develop.

The relevance of the ADH system for the actions of alcohol is less well known than is the case with ALDH. Although seven *ADH* genes have been mapped to chromosome 4 in humans, relevant polymorphism has been found only for *ADH2* and *ADH3* (Smith, 1986). The kinetic differences among ADH2 isozymes are much more striking than those among the ADH3 isozymes. For example, the maximum rate of reaction (V_{\max}) of β_2 (encoded by *ADH2**2) homodimers is around 40 times that of β_1 (encoded by *ADH2**1) homodimers, whereas the V_{\max} of γ_1

From the Department of Mental Health and Alcohol Research (CIPE, T. Sarkola), National Public Health Institute, Helsinki Finland; the Department of Forensic Medicine and Sciences (T. Fukunaga, HY), Mie University School of Medicine, Tsu, Mie, Japan; the Institute of Epidemiology (WJC), College of Public Health, National Taiwan University, Taipei, Taiwan; the Department of Adult Psychiatry (CCC, JMJ), Taipei City Psychiatric Center, Taipei, Taiwan; the Division of Epidemiology and Public Health (ATAC), Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; the University of Applied Science Fulda (KK-M), Fulda, Germany; the Division of Clinical Research (MK, SM, SH), National Institute on Alcoholism, Kurihama National Hospital, Yokosuka, Kanagawa, Japan; Akagi Kougen Hospital (MM), Gunma, Japan; the Department of Neuropsychiatry (HK), Keio University School of Medicine, Tokyo, Japan; the Department of Medicine (LC, T. Stewart, T. Foroud, JS, T-KL), Indiana University School of Medicine, Indianapolis, Indiana; the Foundation for Alcohol Related Research (DV, LB), Cape Town, South Africa; and the Department of Clinical Biochemistry (JBW), Royal Prince Alfred Hospital, Camperdown, Australia.

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Reprint requests: C. J. Peter Eriksson, PhD, Department of Mental Health and Alcohol Research, National Public Health Institute, POB 719, 00101 Helsinki, Finland; Fax: 358-9-1332781; E-mail: peter.eriksson@ktl.fi

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(encoded by *ADH3*1*) homodimers is double that of γ_2 (encoded by *ADH3*2*) homodimers.

The role of the *ADH* polymorphism in the adverse actions of alcohol was first suggested by Stamatoyannopoulos et al. (1975). Preliminary evidence was found in Asian populations by Shibuya et al. (1989), who observed a positive association between flushing and the prevalence of the *ADH2*2* allele. Similar trends or associations have been reported more recently (Chen et al., 1998; Takeshita et al., 1996; Thomasson et al., 1990). In addition, a number of studies have found the *ADH2*2* allele to protect against alcohol abuse and alcoholism in both Asian (Chao et al., 1994, 1997; Chen et al., 1996, 1999; Higuchi, 1994; Higuchi et al., 1996; Maezawa et al., 1995; Muramatsu et al., 1995; Nakamura et al., 1996; Shen et al., 1997; Tanaka et al., 1996; Thomasson et al., 1991, 1994) and white populations (Borras et al., 2000; Neumark et al., 1998; Whitfield et al., 1998). Functional relevance of the *ADH3* gene also has been reported. The *ADH3*1* allele, which is encoding more active enzyme, has been associated with reduced risk for alcohol dependence in Asians (Chao et al., 1994; Chen et al., 1996, 1997; Higuchi et al., 1996; Nakamura et al., 1996; Shen et al., 1997; Thomasson et al., 1991, 1994). However, these rather weak associations have been explained by linkage disequilibrium between *ADH2* and *ADH3* (Borras et al., 2000; Chen et al., 1999; Osier et al., 1999).

The aims of this symposium were to present an overview of the latest knowledge about the functional relevance of ADH in the actions of alcohol. We emphasized methodological tools, whether self-reported flushing involves the contribution of ADH2 and ADH3, how ADH variation may affect alcohol drinking and alcoholism, and the role of ADH in alcohol-related birth defects.

DISCUSSION

Methodological Aspects

The functional relevance of any protein is classically determined by correlating the genotype polymorphism with the relevant functional variability. In contrast to many other correlation studies, the significant correlation in this case naturally would provide strong evidence for causality. The crucial factor that determines the functional relevance of any genetic polymorphism is the phenotype penetrance. In the case of the ADH2 and ADH3, the penetrance of the phenotype with elevated acetaldehyde levels, flushing, and adverse reactions is far less than for the *ALDH2* polymor-

phism. This is because the first step of alcohol oxidation is regulated primarily by the hepatic redox state and to a lesser extent by the ADH activity, and because the acetaldehyde output (i.e., the phenotype) is so sensitive to any change in the ALDH activity. In addition, the studies of *ADH* polymorphism relevance are complicated by the fact that the ADH-mediated alcohol oxidation is regulated by the acetaldehyde level as well.

The fact that there are at least three major polymorphisms (i.e., 27 different genotypes) that should be regarded in any study on the acetaldehyde phenotypes complicates the genetic studies. Especially in studies on Asian populations with common polymorphism at all three genes (*ADH2*, *ADH3*, and *ALDH2*), the ideal study would require great numbers of individuals. For example, a study of the relevance of the *ADH3* polymorphism in Asians who have the *ALDH2*1/*1* and *ADH2*1/*1* genotypes would be very difficult in practical terms. On the other hand, this type of study would be the most suitable within white populations.

Another possibility for future studies on the relevance of the *ADH* polymorphism would be to increase the role of ADH activity in the regulation of alcohol oxidation. This could be achieved by using lower doses of alcohol or by using specific ADH inhibitors. In our laboratory we have used 4-methylpyrazole, which recently was approved by the Food and Drug Administration for the treatment of ethylene glycol poisoning. When an oral dose of 10 to 15 mg/kg was used, the alcohol elimination rate was reduced by 25% to 30%. This demonstrates that 4-methylpyrazole is an excellent tool in the investigation of the general role of ADH in the actions of alcohol in humans. Thus, this 4-methylpyrazole design could be used for human studies on the relative role of the different *ADH* genotypes in the actions of alcohol.

ADH and Flushing in Asians

For the alleles responsible for either faster production (*ADH2*2* and *ADH3*1*) or slower removal (*ALDH2*2*) of acetaldehyde, there are wide variations in their frequencies in various populations (Table 1). Relatively speaking, Japanese and Taiwanese Han are polymorphic in *ADH2* and *ALDH2* but homozygous in *ADH3*, whereas white subjects are homozygous in *ADH2* and *ALDH2* but polymorphic in *ADH3*.

Five studies have examined the relationship between *ALDH2* genotypes and self-reported facial flushing (Table 2). Common features of the studies conducted in Japan were that

Table 1. Allele Frequencies of *ADH2*2*, *ADH3*1*, and *ALDH2*2* Among Asian and White Populations

Allele	Taiwanese aborigines ^a				Han Chinese ^b	Japanese ^c	Malays ^d	Whites ^e
	Atayal	Ami	Bunun	Paiwan				
<i>ADH2*2</i>	0.85	0.70	0.84	0.86	0.73	0.75	0.59	0.04
<i>ADH3*1</i>	0.95	1.00	0.94	0.99	0.95	0.94	—	0.54
<i>ALDH2*2</i>	0.03	0.02	0.02	0.05	0.30	0.24	0.03	0.00

^a Nonalcoholic controls (Chen et al., 1997); ^b nonalcoholic controls (Thomasson et al., 1991); ^c nonalcoholic controls (Higuchi, 1994); ^d unscreened subjects (Goedde et al., 1992); ^e nonalcoholic controls (Gilder et al., 1993).

Table 2. Self-Report of Flushing After Consumption of Alcohol and *ALDH2* Genotype

Authors	Ethnicity/nation	Participants	ALDH2 *1/*1		ALDH2 *1/*2		ALDH2 *2/*2	
			<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Shibuya et al. (1989)	Japanese/Japan	Healthy males	0/5	0.0	7/8	88.0	2/2	100.0
Takeshita et al. (1994)	Japanese/Japan	Nonalcoholic metal workers						
		Males	21/235	8.9	135/160	84.4	27/29	93.1
		Females	5/63	7.9	26/32	81.3	5/5	100.0
Wall et al. (1996b)	Asian descent/U.S.	Nonalcoholic college male students						
		Self-report	9/28	32.0	20/20	100.0	2/2	100.0
		Observed	1/28	4.0	20/20	100.0	2/2	100.0
Chen et al. (1998)	Han/Taiwan	Alcoholic cases and normal controls	14/46	30.0	12/12	100.0	—	—
Takeshita & Morimoto (1999)	Japanese/Japan	University students						
		Males	17/211	8.1	136/157	86.6	18/21	85.7
		Females	0/19	0.0	10/13	76.9	1/2	50.0

the proportion of flushers in subjects with *ALDH2**1/*1 was very low (0–9%), whereas the figure in subjects with *ALDH2**1/*2 was much higher (77–88%). Few subjects were found to be *ALDH2**2/*2, and almost all of them were flushers. In contrast, in the studies of Wall et al. (1996b) and Chen et al. (1998), there were still considerable proportions (around 30%) of flushers in subjects with *ALDH2**1/*1. For subjects with *ALDH2**1/*2 or *ALDH2**2/*2, 100% of them reported flushing after consumption of alcohol. Furthermore, Wall et al. (1996b) found that investigator-observed flushing is more accurate in predicting *ALDH2* genotype than self-reported flushing.

Thus, one remaining issue is whether self-reported flushing among subjects with *ALDH2**1/*1 can be explained by the contribution from *ADH2* or *ADH3*. So far, only two studies have examined specifically the relationship between *ADH2* genotypes and flushing in Asian subjects with *ALDH2**1/*1. In these two studies, both Takeshita et al. (1996) and Chen et al. (1998) found that none of the subjects with *ADH2**1/*1 reported flushing, whereas 8% to 12% of Japanese and 35% to 44% of Taiwanese reported flushing in subjects with either *ADH2**1/*2 or *ADH2**2/*2. Chen et al. (1998) further examined whether the flushing in subjects with *ADH2**1/*2 or *ADH2**2/*2 can be explained by a contribution from *ADH3*. For subjects with *ALDH2**1/*1 and at least one *ADH2**2 allele, the genotype of *ADH3* was not associated with self-reported flushing.

In four of the five studies that examined the relationship between flushing and genotype of alcohol-metabolizing genes, alcoholic subjects were excluded out of concern that tolerance for flushing or liver damage might have occurred after a prolonged period of alcohol drinking. With this in mind, Chen et al. (1998) reminded their subjects in the interview that they were looking for the alcohol sensitivity symptoms at an early stage of alcohol drinking. Such recalling might have reduced the accuracy of the self-report of alcohol sensitivity symptoms in that study.

The previously described results indicated that subjects with *ALDH2**1/*2 whose elimination of acetaldehyde was slow reported to have facial flushing regardless of whether the production of acetaldehyde was fast or slow. Subjects with *ALDH2**1/*1 whose elimination of acetaldehyde was fast still had a 30% probability of reporting facial flushing.

Given the difference in demographic features and alcohol drinking history of subjects between the two studies by Takeshita et al. (1996) and Chen et al. (1998), the similarity in their results indicates a good comparability of self-reported flushing across studies. The results clearly demonstrated that *ADH2**2 can contribute to alcohol-induced flushing for subjects who eliminate acetaldehyde normally.

However, not all subjects with *ALDH2**1/*1 and fast production of acetaldehyde (*ADH2**1/*2 or *ADH2**2/*2) have facial flushing. Will a fast acetaldehyde-producing *ADH3**1 contribute to this? The results of Chen et al. (1998) showed that *ADH3* genotype did not explain the difference in facial flushing for these subjects. This is consistent with the findings that the association of *ADH3* polymorphism with the risk of alcoholism might be due to its linkage disequilibrium with *ADH2*. Other unidentified genetic or environmental factors (such as individual differences in the amount of alcohol intake) may account for this. However, because there is relatively rare polymorphism in *ADH3* in Taiwanese Han, the lack of effect of *ADH3**1 on flushing might be due to insufficient sample size.

Wall et al. (1996b) found that the accuracy of investigator-observed flushing is more accurate than self-reported flushing. For subjects with *ALDH2**1/*2, both investigator-observed and self-report flushing rates were 100%. Among 28 subjects with *ALDH2**1/*1, only 1 (4%) was observed to have flushing. In contrast, 9 (32%) of Wall et al.'s subjects with *ALDH2**1/*1 reported flushing. Despite the differences between self-reported and observed flushing rate in *ALDH2* individuals, we do not believe that the excess in self-reported flushing rate is entirely due to report error or recall bias. Instead, we think that an individual's self-report of flushing may be based more on the experience of different amounts or durations of alcohol intake than on the conditions during the observed alcohol test. Another possibility is that a subject's feeling of flushing might include something other or more than reddening of the skin as observed by an investigator, such as a feeling of heat in the cheeks.

There are two implications of these findings. First, previous studies found that familial resemblance of flushing could not be explained by a single dominant gene. These results clearly showed that both *ALDH2* and *ADH2* con-

tribute to the self-report of flushing. Thus, flushing according to self-reporting is determined by at least two major loci in Taiwanese Han. Second, self-report of low response to alcohol challenge has been found to predict a higher risk for alcoholism among white sons of alcoholics (Schuckit, 1994). Because *ALDH2**2 is not found and *ADH2**2 is rare among white subjects, the individual variation in flushing after alcohol challenge in these subjects could be due to polymorphism of *ADH3*. In other words, in ethnic groups whose genotypes are homozygous in *ALDH2* and *ADH2*, the role of *ADH3**2 may be important in predicting non-flushing and hence associated with an elevated risk for alcoholism.

ADH and Flushing in White Subjects

The association between self-reported flushing and *ADH* and *ALDH* genotypes recently has been studied in white Finnish populations (Yamamoto et al., 1998). The control population consisted of 94 men and 80 women from one working area in Helsinki. Selected volunteers (100 men and 250 women) were recruited through advertisement in different media. The advertisements, in which the flushing reaction was described as an example, focused on the possibility to investigate any unusual effects by alcohol.

The overall polymorphism at the *ADH2* locus is very low in Finland, and consequently only one *ADH2**1/*2 case was found among the controls. However, in the population selected for unusual reactions to alcohol, 13 cases were found. Flushing also was prevalent in this group.

The highest degree of *ADH* polymorphism was found at the *ADH3* locus. On the whole, the overall genotype distribution followed a 1:2:1 pattern in accordance to the Hardy-Weinberg principle. In a closer analysis, it was observed that flushing was more common in men with the *1-alleles than in men with the *2-alleles in both the control and selected populations. Significant allelic differences were not observed in the women. This may be explained by the fact that the women displayed more flushing than the men regardless of genotype. Here the explanation may involve hormonal factors, which have been associated with elevated acetaldehyde levels (Eriksson et al., 1996).

The results demonstrate that flushing may arise as a consequence of both *ADH2* and *ADH3* polymorphism in white populations.

ADH2 and Clinical Characteristics and Disease Course in Asian Alcoholics

The subjects were 357 Japanese alcoholic patients hospitalized in National Kurihama Hospital between June 1991 and December 1992 (324 males, 33 females). The diagnosis of alcoholism was made by DSM-III-R criteria and Feighner's diagnostic criteria. Patients who left the hospital within 4 weeks and those with severe dementia were excluded from this study.

In the patient population, 246 patients had the *ADH2**2

allele (patients were defined as the "superactive group"), and 111 did not have the *ADH2**2 allele ("usual group"). There were no significant differences in sociofamilial backgrounds, such as age, marital status, and years of education in distribution between the usual and superactive groups. Comparisons of psychiatric comorbidities also failed to show significant differences between the two groups, although the frequencies of some disorders, such as mood disorders, substance abuse, and personality disorders, in the usual group tended to be higher than those in the superactive group.

There were no significant differences in the mean age of the first drink, habitual drinking, and drinking more than five units of alcohol per day, but when we compared the age at onset of binge drinking, the usual group experienced binge drinking significantly earlier than the superactive group (40.0 ± 10.8 years vs. 43.4 ± 12.9 years; Student's *t* test, $p = 0.027$). The usual group experienced some withdrawal symptoms significantly earlier than the superactive group, such as finger tremor (39.1 ± 10.4 years vs. 43.7 ± 10.8 years; $p = 0.0012$) and sweating (38.6 ± 10.4 years vs. 43.2 ± 10.3 years; $p = 0.0031$). Other withdrawal symptoms such as insomnia, seizures, hallucinations, and withdrawal delirium showed the same tendency, but they did not reach statistical significance. The frequency of the patients who had experienced sweating in the usual group was higher than that in the superactive group ($p = 0.042$), but there were no differences in the frequencies of other symptoms. The time from the start of habitual drinking to the onset of binge drinking ($p = 0.011$), tremor ($p = 0.0010$), and sweating ($p = 0.0047$) was delayed in the superactive group for several years.

In summary, patients with superactive ADH developed alcohol dependence more slowly than did those with active ADH. This delay resembles the inactive ALDH2-mediated delay in the occurrence of alcohol dependence. These results suggested that superactive ADH delayed development of alcoholism by a mechanism similar to inactive ALDH2-mediated delay in the occurrence of alcohol-related problems and alcoholism. This could involve higher levels of acetaldehyde in the blood produced after ethanol ingestion in the superactive group than in the usual group, which caused adverse reactions that suppressed alcohol dependence.

ADH2 and Alcohol Use in Jewish Populations

Recently, a white Jewish population from Israel was found to have *ADH2**2 allele frequencies of 21% (Neumark et al., 1998), which is far higher than the usual frequency of <5% in white populations (Borras et al., 2000). In addition, those Jewish individuals with the *ADH2**2 allele drank less alcohol than those without the *ADH2**2 allele, which may be part of the explanation for the low rates of alcohol abuse in Jewish compared to other white populations (Neumark et al., 1998). The purpose of this study was to determine if *ADH2* polymorphism influences

alcohol use and/or its consequences in the Jewish populations from Indianapolis.

The sample consisted of 62 college students from Indiana University (average age, 20; range, 18–26) and 90 noncollege subjects who were members of the Indianapolis Jewish Community Center (average age, 49; range, 23–82). All participants were of Ashkenazi descent (Eastern European), and the number of males and females was about equal. The questionnaire was divided into three parts: family background, alcohol use (frequency and quantity), and effects of alcohol. In the questionnaire, one drink was defined as 12 oz. of beer, 4 oz. of wine, or 1 oz. of 80% alcohol.

The *ADH2* allele frequencies were similar between the two groups, but the quantity of alcohol consumed was significantly different. The Jewish general population drank significantly less alcohol than the Jewish college students, 1.4 and 4 drinks per occasion, respectively. Thus, the two groups could not be combined and the data from each group were analyzed separately, by using the Wilcoxon Rank Test.

The general population subjects with an *ADH2*2* allele were more likely to drink alcohol less frequently ($p = 0.03$) and experience more unpleasant effects after one to two alcoholic drinks ($p = 0.06$) than those who did not carry the *ADH2*2* allele. The college subjects with an *ADH2*2* allele were more likely to have unpleasant reactions after one to two alcoholic drinks ($p = 0.01$), to limit their alcohol use due to reactions ($p = 0.05$), and to experience more negative effects after alcohol consumption ($p = 0.003$) compared with the college students who were not *ADH2*2* carriers.

These data suggest that Jewish individuals with the *ADH2*2* allele drink alcohol less frequently and that the heavier drinkers with an *ADH2*2* allele experience unpleasant reactions that limit their alcohol use. These results are in agreement with previous findings that involved Jewish individuals from Israel (Neumark et al., 1998) and Ashkenazi Jewish Americans (Shea et al., 2000).

Etiology of the ADH2 Protection Against Alcohol Drinking in White Subjects

The Alcohol Challenge Twin Study was conducted between 1979 and 1981 with 412 male and female twin subjects age 18 to 34 years (Martin et al., 1985). They consumed 0.75 g/kg of ethanol, and ethanol concentrations were determined over the following 3 hr in blood and in breath. Blood samples from these subjects in 1991 to 1994 provided *ADH2* genotypes on 377 subjects (Whitfield et al., 1998), and interview data established the presence or absence of lifetime alcohol dependence by DSM-III-R criteria (Heath et al., 1997) on 334 of them. Data analysis was performed mainly by using Mx (Neale, 1999), which allows for the twin structure of the study sample but imposes some

restrictions on inclusion of twin pairs with only one participating subject, or subjects with incomplete data.

Subjects who had the *ADH2*1/*2* genotype had lower sex-adjusted blood alcohol concentrations after the alcohol challenge than *ADH2*1/*1* subjects; there were no subjects with the *ADH2*2/*2* genotype. However, the results showed similar differences between groups at all six times of measurement, rather than the expected similar peak values followed by divergence of the results. When the results were analyzed by a repeated-measures analysis of variance, without allowing for the fact that the subjects were twins, a significant difference could be shown; but the analysis that used Mx (on smaller numbers of subjects because of the requirement for complete data) showed only a marginally significant *ADH2* effect. Further data analysis is in progress to determine whether the difference is significant.

Comparison of blood alcohol results between subjects who did or did not meet the criteria for alcohol dependence also showed an association, with alcohol-dependent subjects having higher blood alcohol values at all times after alcohol challenge. Conversely, the probability of alcohol dependence increased across quartiles of blood alcohol concentration, with the risk (relative to the lowest quartile) being around three times as great for men in the top quartile and twice as great for women in the top quartile. Multivariate analysis that incorporated sex, *ADH2* genotype, smoking status and habitual alcohol intake at the time of alcohol challenge, and lifetime alcohol dependence showed that dependence status had a significant association with blood alcohol concentration. Once again the difference in blood alcohol concentrations was similar across different times after alcohol ingestion, rather than the values diverging as postabsorptive metabolism proceeded.

These results test the hypothesis that *ADH2* variation affects alcohol dependence risk through variation in alcohol metabolism or alcohol pharmacokinetics. On the whole, the results are consistent with the hypothesis, although the fact that the differences in blood alcohol are essentially constant across time is unexpected. It appears that the differences between *ADH2*1/*1* and *ADH2*1/*2* subjects, or between alcohol-dependent and nondependent subjects, have their origin in preabsorptive or first-pass metabolism in the stomach or in the liver. Because gastric metabolism is relatively slight in fasting subjects or after substantial doses of alcohol, we need to consider whether variation in hepatic ADHs can account for variation in early alcohol metabolism, perhaps through differences in *K_i* for NADH between the polymorphic forms of *ADH2*.

Previous literature on *ADH2* genotype and in vivo ethanol metabolism has shown either small effects of *ADH2*3* (Thomasson et al., 1995) or no significant effect (Wall et al., 1996a). *ADH2*2* was found to have no effect on the postabsorptive rate of decrease in blood alcohol (Mizoi et al., 1994), but information on the peak alcohol concentrations was lacking. In relationship to the difference in de-

pendence risk by blood alcohol concentration, there seem to have been no previous studies.

To summarize, the current results are consistent with the acetaldehydogenic hypothesis of ADH2 effects, but gaps still remain, particularly because of the lack of acetaldehyde concentration data. Because alcohol metabolism seems to be a risk factor for alcohol dependence, further research must include a search for genes that affect alcohol metabolism and tests for their effects on dependence.

ADH2 and Birth Defects

Development of fetal alcohol syndrome (FAS) after excessive alcohol exposure is due, in part, to genetic factors. A mixed ancestry population from western Cape Town of South Africa, where there is a high degree of FAS, was studied to determine whether ADH2 polymorphism influenced the risk for FAS. FAS children were identified by screening 7-year-old school entrants. The children who were lower than the tenth percentile in height, weight, and occipital facial circumference were further evaluated by the Griffiths test, a neurodevelopmental assessment. The mothers of the children were interviewed, and a history of their alcohol use during pregnancy was obtained. A consensus FAS diagnosis was established by the clinicians, maternal interviewers, and neurodevelopmental pediatric specialists; the FAS rate in this population was determined to be 6.4%.

The sample for this study consisted of 41 FAS children and their mothers (41) and 178 control individuals of mixed ancestry from the same rural geographic region as the FAS children and their mothers. The *ADH2*2* allele frequencies of the mothers and FAS children were compared with the control group by using a one-sided *Z* test to determine whether the *ADH2*2* allele had a protective effect against FAS. A Bonferroni correction for multiple testing ($n = 2$) was applied, and only those comparisons that generated a *p* value < 0.0253 were considered significant at the conventional 0.05 level. The FAS children and the FAS mothers had a significantly lower frequency of the *ADH2*2* allele compared with the control group ($p = 0.025$ and 0.01, respectively). Thus, the *ADH2*2* allele appears to have a protective effect against FAS in this population.

The mechanism of how *ADH2*2* might protect against FAS is not known; however, *ADH2*3*, also a high-activity ADH, has been shown to be protective against fetal alcohol effects (McCarver et al., 1997). It may be that mothers with the high-activity ADHs drink less alcohol than mothers with the low-activity ADH, or blood ethanol levels may be lower in mothers who have the *ADH2*2* allele because the high-activity enzymes more efficiently metabolize alcohol at high blood alcohol concentrations.

SIGNIFICANCE

It can be concluded that the *ADH2*2* allele contributes to the flushing and to the biological protection against

alcohol abuse in both Asian and white populations. Although the role of *ALDH2* polymorphism in Asians is more pronounced compared with *ADH2*, the overall significance may be of the same order due to the larger global prevalence of the *ADH2*2* allele. We also should emphasize that the *ADH2* polymorphism could be of decisive importance in Asian *ALDH2*1*2* individuals as well.

As a consequence of the protective effect of the *ADH2*2* allele against alcohol abuse, the frequency of harmful effects is also lower in these individuals on a general population basis. However, this form of indirect protection doesn't exclude the possibility that the same allele would, in fact, have a detrimental effect in the pathological actions of alcohol in those individuals who abuse the alcohol. Thus, the concept of protection is context-dependent.

In contrast to the role of *ADH2*, the significance of *ADH3* polymorphism seems to be low, and part of the data may be explained by linkage disequilibrium with *ADH2*. On the other hand, the association between flushing and the *ADH3*1* allele in the Finnish population demonstrates that the overall significance of the *ADH3* polymorphism needs to be settled in future studies. One aspect that may complicate investigations of the role of the *ADH3* polymorphism, as well as the role of *ADH2*, is the possibility of dual mechanisms. It has been suggested that acetaldehyde may have both adverse effects that inhibit alcohol drinking and euphoric effects (by separate mechanism) that promote alcohol drinking (Eriksson, 1982). If this were the case, we would have to reconsider the significance of all the *ADH* polymorphisms.

The mechanism of the actions of ADH effects is believed to involve acetaldehyde production (determined by the rate of alcohol oxidation). This hypothesis is supported by the fact that the same adverse effects and the protection against alcohol abuse are caused by either inhibition of acetaldehyde oxidation or acceleration of alcohol oxidation. The problem here is the lack of metabolic data to support the hypothesis. Thus, data on the association between *ADH* polymorphism and the functional alcohol and acetaldehyde metabolism are needed before the final significance is revealed.

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