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Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai

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Abstract Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), the principal enzymes responsible for oxidative metabolism of ethanol, exist in multiple, genetically determined molecular forms. Widely different kinetic properties in some of these isozymes account for the individual differences in alcohol sensitivity. In this study we used the polymerase chain reaction/restriction fragment length polymorphism method to determine the genotypes of the ADH2 and ALDH2 loci of alcoholic and nonalcoholic Chinese living in Shanghai. We also investigated the subjects' drinking patterns by means of semi-structured interviews. The alcoholics had significantly lower frequencies of the $ADH2^2$ and $ALDH2^2$ alleles than did the nonalcoholics, suggesting the inhibitory effects of these alleles for the development of alcoholism. In the nonalcoholic subjects, $ADH2^2$ had little, if any, effect, despite the significant effect of the $ALDH2^2$ allele in decreasing the alcohol consumption of the individual. Taken together, these results fit the proposed hypothesis for the development of alcoholism, i.e., drinking behavior is greatly influenced by the individual's gentoypes of alcohol-metabolizing enzymes, and the risk of becoming alcoholic is proportionate with the ethanol consumption of the individual.

Introduction

Most ethanol elimination occurs by oxidation to acetaldehyde and acetate, catalyzed by alcohol dehydrogenase (ADH) and mitochondrial aldehyde dehydrogenase (ALDH2). The oxidation of ethanol by ADH is performed by homodimeric and heterodimeric isozymes whose subunits are encoded by the ADH1, ADH2, and ADH3 genes. Of special interest is the ADH2 isozyme, which is polymorphic in diverse ethnic groups and which can have different kinetic properties (Yoshida et al. 1991). The $ADH2^2$ allele is highly prevalent among Orientals. The $\beta_2\beta_2$ isozyme encoded by this allele has a very high V_{max} for ethanol, and hence is called "superactive."

Genetic polymorphism also occurs at the ALDH2 gene locus, which encodes an inactive subunit with a single point mutation corresponding to an amino acid substitution (Yoshida et al. 1984). The inactive $ALDH2^2$ allele is dominant; the enzyme in individuals who have this allele has little or no activity (Crabb et al. 1989).

The $ALDH2^2$ allele is relatively common in Orientals. A well-established association exists between the inactive ALDH2 and "Oriental flushing response" characterized by facial flushing and other symptoms, such as headache, tachycardia, and nausea because of high blood acetaldehyde concentration following ethanol ingestion (Harada et al. 1981). This unpleasant response has been considered to be a genetic deterrent to heavy drinking and alcoholism among Orientals (Harada et al. 1982). Recent studies suggest that $ADH2^2$ might also have a protective effect against alcoholism (Thomasson et al. 1991; Higuchi 1995). One can assume from these results that both the ADH2 and ALDH2 alleles could affect drinking behavior in healthy populations. Until recently, however, there has only been one study, from Japan, concerning this problem; this demonstrated that only the ALDH2 genotypes affect drinking behavior (Takeshita et al. 1994). These results raise the question as to whether the same association can be found in other populations with different cultural backgrounds. We therefore examined the effects of both ADH2 and ALDH2 allelic differences on drinking behavior and risk for alcoholism among Chinese living in Shanghai. Although little is known about drinking behavior in the People's Republic of China, it is believed that alcohol-related problems increase in parallel with eco-

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Table 1 Genotype and allele frequencies of ADH2 and ALDH2

Subject group	Genotype frequency			Allele frequency	
	$ADH2^1/ADH2^1$	$ADH2^1/ADH2^2$	$ADH2^2/ADH2^2$	$ADH2^1$	$ADH2^2$
Nonalcoholics (n = 105)	0.11	0.41	0.48	0.32	0.68
Alcoholics (n = 32)	0.41	0.25	0.34	0.53	0.47[a]
	Genotype frequency			Allele frequency	
	$ALDH2^1/ALDH2^1$	$ALDH2^1/ALDH2^2$	$ALDH2^2$ $ALDH2^2$	$ALDH2^1$	$ALDH2^2$
Nonalcoholics (n = 105)	0.54	0.41	0.05	0.75	0.25
Alcoholics (n = 32)	0.91	0.09	0.00	0.95	0.05[b]

[a] $\chi^2 = 9.49$, $P < 0.005$
[b] $\chi^2 = 13.80$, $P < 0.001$

nomic development. Given the recent economic growth in China, a survey in a city such as Shanghai is a very valuble step towards predicting and preventing any future alcohol-related problems in this vast country.

Subjects and methods

The nonalcoholic subjects in this study were 105 Chinese (63 men and 42 women, ages 19–65 years; mean, 39.8 ± 11.8 for men, 34.5 ± 10.4 for women) living in Shanghai, most of them hospital employees. Information on drinking behavior was obtained by one of the authors in face-to-face semistructured interviews. The questionnaire used was the Chinese version of that originally developed at the Japanese National Institute on Alcoholism (Higuchi et al. 1992).

The Chinese alcoholic subjects were inpatients of the Shanghai Mental Health Center. All were men who met the DSM-III-R criteria for alcohol dependence.

After informed consent had been obtained, a blood sample was obtained from each subject. DNA was extracted from the buffy coat by a standard method, and ADH2 and ALDH2 genotyping were performed by the polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method (Xu et al. 1988; Harada 1992). Briefly, portions of exon 3 of the ADH2 gene were amplified by using PCR, digested with *Mae*III, electrophoresed on 2% agarose gel, and viewed with the aid of ethidium bromide staining. Similarly, portions of exon 12 of ALDH2 were digested with *Mbo*II after amplification, electrophoresed on 12% acrylamide gel, and viewed.

Results

The genotype and allele frequencies of the ADH2 and ALDH2 loci are shown in Table 1. The $ADH2^2$ and $ALDH2^2$ alleles were present with a significantly lower frequency among alcoholics than among nonalcoholics. These results generally agreed with those of an earlier study of Chinese living in Taiwan by Thomasson et al. (1991).

The relationship between the presence of the ADH2 genotype and the drinking patterns of nonalcoholic male subjects is shown in Table 2. The $ADH2^2/ADH2^2$ homozygotes appeared to drink less often and to consume less alcohol per month, although none of these differences reached statistical significance. All of the female subjects drank less than 25 ml pure alcohol per occasion, and their average monthly alcohol consumption was significantly lower than that of males (6.6 ml vs 188.6 ml); thus, women were considered a uniform group in terms of drinking behavior. Indeed, there were no significant differences between the drinking patterns of women with any of the ADH2 or ALDH2 genotypes (data not shown).

The relationship between the ALDH2 genotype and drinking patterns is presented in Table 3. All $ALDH2^2/ALDH2^2$ homozygotes drank less than once a week, and the amount that each individual drank per occasion was

Table 2 Drinking patterns of nonalcoholic male subjects, by ADH2 genotype

	$ADH2^1/ADH2^1$ n = 8	$ADH2^1/ADH2^2$ n = 27	$ADH2^2/ADH2^2$ n = 28
Drinking frequency			
< 1 day/week	4 (50.0%)	17 (61.5%)	20 (71.4%)
≥ 1 day/week	4 (50.0%)	10 (38.5%)	8 (28.6%)
Consumption per occasion (average, pure ethanol)			
< 50 ml	7 (87.5%)	18 (66.7%)	22 (78.6%)
≥ 50 ml	1 (12.5%)	9 (33.3%)	6 (21.4%)
Consumption per month (average, pure ethanol)	433 ml	173 ml	132 ml

Table 3 Drinking patterns of nonalcoholic male subjects, by ALDH2 genotype

	$ALDH2^1/ALDH2^1$ n = 31	$ALDH2^1/ALDH2^2$ n = 29	$ALDH2^2/ALDH2^2$ n = 3
Drinking frequency			
< 1 day/week	20 (64.5%)	18 (62.1%)	3 (100.0%)
≥ 1 day/week	11 (35.5%)	11 (37.9%)	0 (0.0%)
Consumption per occasion (average, pure ethanol)			
< 50 ml	19 (61.3%)	25 (86.2%)	3 (100.0%)
≥ 50 ml	12 (38.7%)	4 (13.8%)	0 (0.0%)
Consumption per month (average, pure ethanol)	201 ml	193 ml	7 ml[a]

[a] $P < 0.005$ vs $ALDH2^1/ALDH2^2$; $P < 0.001$ vs $ALDH2^1/ALDH2^1$

less than 50 ml pure ethanol. Moreover, the average monthly alcohol consumption by the $ALDH2^2/ALDH2^2$ homozygotes was significantly lower than the amount of alcohol consumed by the other subjects (*t*-test).

Discussion

The genetic variability at the human ADH2 and ALDH2 loci has been considered a potential explanation for individual differences in drinking behavior. In this study of Chinese living in Shanghai, we have demonstrated the relationships between the $ADH2^2$ and $ALDH2^2$ alleles and the drinking patterns of nonalcoholics, and the allelic distribution among alcoholics. China's largest city, Shanghai, has a population of 13 million, and is a major commercial and manufacturing center. It is therefore a good city for sampling studies to predict the future of the economically growing nation.

The recently developed PCR-RFLP method for genotyping ADH2 and ALDH2 used in this survey is simple and reliable for this type of study. We have previously confirmed its reliability by comparing the results obtained by PCR-RFLP with those obtained via the allele-specific oligonucleotide hybridization method (Higuchi 1995).

The allelic distribution of ADH2 and ALDH2 in alcoholics and nonalcoholics shows the inhibitory effect of not only $ALDH2^2$ alleles, but also $ADH2^2$ alleles in the development of alcoholism. That not a single case with $ALDH2^2/ALDH2^2$ could be found among the Chinese alcoholics indicates the stronger inhibitory effect of this allele, which is consistent with earlier findings in Taiwan and Japan (Thomasson et al. 1991; Higuchi 1995).

Blood acetaldehyde levels after drinking differ among individuals with the three ALDH2 genotypes (Yamamoto et al. 1993). Accordingly, drinking patterns should differ among the three genotypes if blood acetaldehyde is the major determining factor for drinking behavior. Our results demonstrate that the amount of drinking in males decreases according to the sequence $ALDH2^1/ALDH2^1 > ALDH2^1/ALDH2^2 > ALDH2^2/ALDH2^2$, drinking by individuals with $ALDH2^2/ALDH2^2$ being conspicuously lower, which is consistent with the findings recorded in the challenging study of Yamamoto et al. (1993). Their research showed that $ALDH2^2/ALDH2^2$ homozygotes had blood acetaldehyde concentrations approximately 19 and 3 times those of $ALDH2^1/ALDH2^1$ homozygotes and $ALDH2^1/ALDH2^2$ heterozygotes, respectively.

Genotype-specific differences in the kinetic properties of ADH2 have been reported. Under predicted physiological conditions, the $\beta_2\beta_2$ isozyme encoded by $ADH2^2$ oxidizes ethanol 20 times as fast as does the $\beta_1\beta_1$ isozyme encoded by $ADH2^1$ (Bosron and Li 1988). Moreover, the V_{max} of $\beta_2\beta_2$ is 40-fold that of the $\beta_1\beta_1$ isozyme (Bosron and Li 1986). Thus, individuals with $ADH2^2$ might be expected to generate acetaldehyde more rapidly after drinking alcohol than would those with only $ADH2^1$, and this would thereby affect their drinking behavior. Our results have, however, revealed no significant differences among males with the three genotypes, suggesting that any effect the ADH2 allele has on drinking behavior is smaller than that of the ALDH2 allele.

Among women, we found no differences in drinking patterns between different ADH2 or ALDH2 genotypes. This is probably attributable to their low alcohol consumption. Traditionally in China, alcohol drinking has been an accepted behavior for males, but not for females.

Taken together, these results support the notion that the development of alcoholism depends, at least in part, upon the amount of drinking, which in turn is substantially influenced by the genetically determined properties of the alcohol-metabolizing enzymes. Our study in the People's Republic of China shows that $ADH2^2$ and $ALDH2^2$ serve as genetic deterrents for alcoholism, but that the effect of $ADH2^2$ on drinking behavior in nonalcoholics is smaller than that of $ALDH2^2$. These results, which are all consistent with those of earlier studies conducted in Japan and Taiwan, suggest that the role of these genes in individual drinking behavior is essentially the same in different cultures.

In Japan, where the allelic distributions of ADH2 and ALDH2 are similar, but where alcohol consumption is higher than in China, alcohol-related problems constitute one of the biggest issues in public health, burdening the nation with a social cost of more than $ 60000 million a year (Nakamura et al. 1993). Because of the conspicuous interindividual differences in alcohol sensitivity among the population, a genotype-specific prevention program has been proposed in Japan. The data obtained in this study in Shanghai, i.e., the similar genotype effect on drinking behavior, could also prove valuable in planning preventive strategies for alcohol-related problems in the People's Republic of China.

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