Am. J. Hum. Genet. 48:677–681, 1991

Alcohol and Aldehyde Dehydrogenase Genotypes and Alcoholism in Chinese Men

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Summary

The liver enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are responsible for the oxidative metabolism of ethanol, are polymorphic in humans. An allele encoding an inactive form of the mitochondrial ALDH2 is known to reduce the likelihood of alcoholism in Japanese. We hypothesized that the polymorphisms of both ALDH and ADH modify the predisposition to development of alcoholism. Therefore, we determined the genotypes of the *ADH2, ADH3,* and *ALDH2* loci of alcoholic and nonalcoholic Chinese men living in Taiwan, using leukocyte DNA amplified by the PCR and allele-specific oligonucleotides. The alcoholics had significantly lower frequencies of the *ADH2*2, ADH3*1,* and *ALDH2*2* alleles than did the nonalcoholics, suggesting that genetic variation in both ADH and ALDH, by modulating the rate of metabolism of ethanol and acetaldehyde, influences drinking behavior and the risk of developing alcoholism.

Introduction

Most ethanol elimination occurs by oxidation to acetaldehyde and acetate, catalyzed principally by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). There are multiple isozymes of ADH and ALDH in human liver (Bosron and Li 1986; Smith 1986). The ADHs primarily involved in hepatic ethanol metabolism are the homo- and heterodimeric isozymes whose subunits are encoded by the *ADH1, ADH2,* and *ADH3* genes (Bosron and Li 1986; Smith 1986) closely linked on chromosome 4 (Smith 1986; Tsukahara and Yoshida 1989; Yasunami et al. 1990). Polymorphic alleles at the *ADH2* (β-subunit) and *ADH3* (γ-subunit) loci encode isozymes that differ strikingly in catalytic properties (Bosron and Li 1986). These differences are thought to underlie a part of the threefold variation in alcohol elimination rates among individuals (Bennion and Li 1976; Wagner et al. 1976), of which 50% is thought to be genetic in origin (Kopun and Propping 1977; Martin et al. 1985). Oxidation of acetaldehyde to acetate is believed to be catalyzed primarily by ALDH2, the low-K_m form of ALDH in mitochondria (Smith 1986). The gene for this homotetrameric enzyme is situated on chromosome 12 (Hsu et al. 1986). A point mutation in the *ALDH2* gene produces a deficiency in ALDH2 activity (Yoshida et al. 1984; Hsu et al. 1988). The mutant allele *ALDH2*2* is dominant over the normal *ALDH2*1* allele; persons both homozygous and heterozygous for *ALDH2*2* lack detectable ALDH2 activity in liver (Crabb et al. 1989; Goedde et al. 1989). ALDH2 deficiency is relatively common among Asians (Goedde et al. 1979; Harada et al. 1980; Teng 1981; Smith 1986). It is associated with facial flushing and other unpleasant symptoms, such as light-headedness, palpitations, and nausea, when alcohol is consumed (Wolff 1972). This alcohol-induced flush reaction is very similar to the aversive reaction caused by alcohol ingestion in patients being treated with the ALDH in-

Received July 16, 1990; revision received October 2, 1990.

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0002-9297/91/4804-0005$02.00

hibitor disulfiram (Ritchie 1980) and is associated with elevated levels of blood acetaldehyde (Mizoi et al. 1979, 1985; Tsukamoto et al. 1989). The ALDH2-deficient phenotype is much less common in Japanese alcoholics (Harada et al. 1982, 1983, 1985; Yoshihara et al. 1983) than in the Japanese population in general.

ALDH2 deficiency presumably lowers the risk of alcoholism as a result of slow acetaldehyde removal. Since differences in ADH activity can affect the rate of acetaldehyde production, we hypothesized that the frequency of *ADH2* and *ADH3* alleles will also be different in alcoholics and nonalcoholics.

Material and Methods

Chinese alcoholic subjects, alcohol dependent by DSM-III criteria (American Psychiatric Association 1980), were male patients from the Tri-Service General Hospital in Taipei. The nonalcoholic Chinese subjects were males from among the students, physicians, and laboratory staff of the National Defense Medical Center. Informed consent was obtained, and blood was drawn from each subject. Genomic DNA was prepared (Madisen et al. 1987), and portions of exons 3 and 9 of the *ADH2* gene and of exon 8 of the *ADH3* gene were amplified by a minor modification of the PCR method described by Xu et al. (1988), which allowed amplification of all three exons in a single reaction. Exon 12 of the *ALDH2* gene was amplified by using PCR as reported by Crabb et al. (1989). *ADH* alleles were distinguished by using allele-specific oligonucleotides (Xu et al. 1988) as probes to hybridize amplified DNA fixed to nitrocellulose. *ALDH* alleles were also distinguished by allele-specific oligonucleotide probes (Crabb et al. 1989) by using buffers containing tetramethylammonium chloride (DiLella and Woo 1987).

Differences in genotypes and allele frequencies were tested for significance by using the χ^2 test. Calculations were carried out using Statview I™ on a Macintosh computer.

Results

The genotypes at the *ADH2, ADH3,* and *ALDH2* loci were determined by means of allele-specific oligonucleotide hybridization after amplifying the relevant segments of the genes by the PCR (Xu et al. 1988; Crabb et al. 1989). The *ADH2* and *ADH3* allele frequencies in the nonalcoholic group agreed with the isozyme patterns in Chinese from Malaysia, which were determined from lung specimens for *ADH2* (Lee et al. 1989) and from stomach tissue for *ADH3* (Teng et al. 1979). The *ALDH2* allele frequencies were similar to those reported to occur among the Japanese (Shibuya and Yoshida 1988).

There were striking differences between the alcoholics and the nonalcoholics, in both the genotype and allele frequencies, at all three loci examined (table 1). The *ADH2*2, ADH3*1,* and *ALDH2*2* alleles were all significantly less frequent among alcoholics than among nonalcoholics ($P < .005$ for each allele).

The *ALDH2*2* allele is dominant: both homozygotes and heterozygotes are phenotypically ALDH2 deficient (Crabb et al. 1989; Goedde et al. 1989). There is a significant difference ($P < .0001$) between alcoholics and nonalcoholics in the predicted ALDH2 phenotype frequencies: 48% of the nonalcoholics but only 12% of the alcoholics have at least one *ALDH2*2* allele and are, therefore, predicted to be deficient in ALDH2 activity. This agrees well with the frequency of ALDH2 deficiency in other Asian groups (Shibuya and Yoshida 1988; Goedde et al. 1989).

To determine whether the effects of the *ADH2* and *ADH3* genotypes were independent of the *ALDH2* genotype, the subgroups containing individuals homozygous for the *ALDH2*1* allele were compared (table 2). All these individuals are predicted to have normal ALDH2 activity. Among these subjects, the differences between alcoholics and nonalcoholics in the frequencies of both *ADH2*2* and *ADH3*1* alleles remained significant ($P < .03$).

Discussion

The present paper is the first report of a significant difference in *ADH2* and *ADH3* genotypes between alcoholics and nonalcoholics. Until now, the deficiency of mitochondrial ALDH2 was the only defined genetic factor known to affect the risk of developing alcoholism (Harada et al. 1982). A report of an allelic association of the human dopamine D_2 receptor gene with alcoholism appeared recently (Blum et al. 1990). Although this dopamine receptor subtype has been implicated in mediating reward in the limbic circuitry of brain (Koob and Bloom 1988), the functional significance of the allelic difference, if confirmed, is unknown.

Alcoholics have significantly lower frequencies of both *ADH2*2* and *ADH3*1* alleles than do nonalcoholics from the same population in Taiwan (table 1).

Table 1

***ADH* and *ALDH*: Genotype Frequencies and Allele Frequencies**

Group (N[a])	Genotype Frequency[b]			Allele Frequency	
	*ADH2*1/*1*	*ADH2*1/*2*	*ADH2*2/*2*	*ADH2*1*	*ADH2*2*
Nonalcoholics (47)	.06	.40	.53	.27	.73
Alocholics (49).....	.37[c]	.31[c]	.33[c]	.52[d]	.48[d]
	Genotype Frequency[b]			Allele Frequency	
	*ADH3*1/*1*	*ADH3*1/*2*	*ADH3*2/*2*	*ADH3*1*	*ADH3*2*
Nonalcoholics (47)	.89	.11	.00	.95	.05
Alcoholics (49).....	.61[d]	.33[d]	.06[d]	.78[d]	.22[d]
	Genotype Frequency[b]			Allele Frequency	
	*ALDH2*1/*1*	*ALDH2*1/*2*	*ALDH2*2/*2*	*ALDH2*1*	*ALDH2*2*
Nonalcoholics (50)	.52	.36	.12	.70	.30
Alcoholics (50).....	.88[d]	.12[d]	.00[d]	.94[d]	.06[d]

[a] Number of individuals in group. Note that some exons did not amplify well or gave ambiguous results; thus some individuals were excluded.

[b] Fraction of group with each genotype; because of rounding errors, some groups' frequencies do not sum to 1.00.

[c] Alcoholics are significantly different from nonalcoholics ($P < .002$). The *ADH2* genotype distribution among alcoholics did not fit the Hardy-Weinberg equilibrium; all other genotype distributions did.

[d] Alcoholics are significantly different from nonalcoholics ($P < .005$).

This difference is independent of the *ALDH2* genotype, as demonstrated by comparison of the groups homozygous for the *ALDH2*1* allele (table 2). This indicates that the *ADH2* and *ADH3* alleles affect the propensity for alcoholism. *ADH2* and *ADH3* are closely linked on chromosome 4 (Smith 1986; Tsukahara and Yoshida 1989; Yasunami et al. 1990). Among the alcoholics homozygous for *ADH2*2*, the *ADH3*1* allele frequency is not significantly different than that among the total population of nonalcoholics. Among the alcoholics homozygous for *ADH2*1*, the *ADH3*2* allele frequency is significantly higher ($P < .001$) than that in the nonalcoholic population. Thus, the *ADH3*2* allele appears to be accompanying the *ADH2*1* allele. A smaller study that compared *ADH2* genotypes in nonalcoholics with those in Japa-

Table 2

ALH* and *ALDH*: Genotype Frequencies and Allele Frequencies among Individuals Homozygous for *ALDH2*1

Group (N[a])	Genotype Frequency[b]			Allele Frequency	
	*ADH2*1/*1*	*ADH2*1/*2*	*ADH2*2/*2*	*ADH2*1*	*ADH2*2*
Nonalcoholics (25)	.08[c]	.48[c]	.44[c]	.32[c]	.68[c]
Alcoholics (43).....	.37[d]	.28[d]	.35[d]	.51[d]	.49[d]
	Genotype Frequency[b]			Allele Frequency	
	*ADH3*1/*1*	*ADH3*1/*2*	*ADH3*2/*2*	*ADH3*1*	*ADH3*2*
Nonalcoholics (25)	.84[c]	.16[c]	.00[c]	.92[c]	.08[c]
Alcoholics (43).....	.58[d]	.35[d]	.07[d]	.76[d]	.24[d]

[a] Number of individuals in group.

[b] Fraction of group with each genotype.

[c] Nonalcoholics homozygous for *ALDH2*1* were not significantly different from nonalcoholics who have an *ALDH2*2* allele.

[d] Alcoholics are significantly different from nonalcoholics ($P < .03$).

nese patients with alcoholic liver disease showed no difference in *ADH2* allele frequencies (Shibuya and Yoshida 1988). Because only 10%–16% of alcoholics develop liver disease (Klatskin 1961; Sorensen et al. 1984), they may not be genotypically representative of the alcoholic population in general.

Our study also demonstrates a difference in *ALDH2* genotype between alcoholics and nonalcoholics among the Chinese men in Taiwan. The *ALDH2*2* allele frequency and the *ALDH2*2/*2* and *ALDH2*1/*2* genotypes that predict phenotypic ALDH2 deficiency are significantly lower in the Chinese alcoholic group than in the nonalcoholics (table 1). Our findings for the Chinese are consistent with reports of lower frequencies of ALDH2 deficiency (Harada et al. 1982, 1983, 1985; Yoshihara et al. 1983) and of a lower frequency of the *ALDH2*2* allele (Shibuya and Yoshida 1988) in Japanese alcoholics, as compared with nonalcoholics.

All the *ADH2* and *ALDH2* alleles found to be at lower frequencies in alcoholics produce isozymes that are predicted to elevate acetaldehyde levels at least transiently. The $\beta_2\beta_2$ isozyme encoded by *ADH2*2* has a 40-fold higher V_{max} than does the $\beta_1\beta_1$ isozyme encoded by *ADH2*1* (Bosron and Li 1986). Under predicted physiologic conditions, $\beta_2\beta_2$ enzymes oxidize ethanol 20-fold faster than do $\beta_1\beta_1$ enzymes (Bosron and Li 1988). The $\gamma_1\gamma_1$ isozyme, encoded by *ADH3*1*, has a V_{max} about twice that of $\gamma_2\gamma_2$, encoded by *ADH3*2*. The heterodimeric ADH isozymes (e.g., $\beta_1\gamma_1$) display kinetic properties intermediate between the corresponding homodimers. Individuals possessing the *ADH2*2* and *ADH3*1* alleles should, therefore, generate acetaldehyde more rapidly after ethanol consumption than do individuals with only the *ADH2*1* and *ADH3*2* alleles. A study of eight Japanese men who flushed on consumption of alcohol and of six who did not do so found that the *ADH2*1* allele was less frequent among flushers (.06) than among nonflushers (.25). Although the sample size was so small that the difference was not statistically significant (Shibuya et al. 1989), the result is consistent with our hypothesis. As with ALDH2 deficiency, which slows the elimination of acetaldehyde, higher acetaldehyde levels generated by the more active ADH isozymes should deter heavy drinking. Since the kinetic differences among the *ADH2*-encoded β isozymes are much more striking than those between the *ADH3*-encoded γ isozymes, we expect that the differences arising from the *ADH2* alleles play the larger role in affecting the risk for alcoholism.

The simplest explanation of the significantly lower frequency of *ADH2*2*, *ADH3*1*, and *ALDH2*2* alleles among alcoholic men in Taiwan is that each can produce higher transient levels of acetaldehyde, through either faster production or slower removal, and that even transient elevation of acetaldehyde may trigger aversive reactions. These aversive reactions may make people with these alleles less likely to become alcoholics. This extends the earlier hypothesis explaining the relatively low frequency of alcoholics with *ALDH2* deficiency (Goedde et al. 1979; Teng 1981; Harada et al. 1982) to a mechanism for the effects of the ADH genes.

Acknowledgments

This work was supported by National Institute on Alcohol Abuse and Alcoholism grants AA06460 (to H.J.E.), AA06434 (to D.W.C.), AA02342 (to T.-K.L.), and AA07611 (to T.-K.L.), by the Alcoholic Beverages Medical Research Foundation grants (to D.W.C. and H.R.T.), and by a National Science Council of the Republic of China grant (to S.J.Y.). A portion of these data has been presented in abstract form in *Clinical Research* (4:898A, 1989).

References

American Psychiatric Association (1980) Diagnostic and statistical manual of mental disorders, 3d ed. American Psychiatric Association, Washington, DC, pp 169–170

Bennion LJ, Li T-K (1976) Alcohol metabolism in American Indians and whites. N Engl J Med 294:9–13

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, et al (1980) Allelic association of human dopamine D_2 receptor gene in alcoholism. JAMA 263:2055–2060

Bosron WF, Li T-K (1986) Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. Hepatology 6:502–510

——— (1988) Catalytic and structural properties of the human liver γγ alcohol dehydrogenase isoenzymes. In: Kuriyama K, Takada A, Ishii H (eds) Biomedical and social aspects of alcohol and alcoholism. Elsevier Science, Amsterdam, pp 31–34

Crabb DW, Edenberg HJ, Bosron WF, Li T-K (1989) Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. J Clin Invest 83:314–316

DiLella A, Woo S (1987) Hybridization of genomic DNA to oligonucleotide probes in the presence of tetramethylammonium chloride. Methods Enzymol 152:447–451

Goedde HW, Harada S, Agarwal DP (1970) Racial differences in alcohol sensitivity: a new hypothesis. Hum Genet 51:331–334

Goedde HW, Singh S, Agarwal DP, Fritze G, Stapel K, Paik YK (1989) Genotyping of mitochondrial aldehyde dehydrogenase in blood samples using allele-specific oligonucleotides: comparison with phenotyping hair roots. Hum Genet 81:305–307

Harada S, Agarwal DP, Goedde HW (1985) Aldehyde dehydrogenase polymorphism and alcohol metabolism in alcoholics. Alcohol 2:391–392

Harada S, Agarwal DP, Goedde HW, Ishikawa B (1983) Aldehyde dehydrogenase isozyme variation and alcoholism in Japan. Pharmacol Biochem Behav 18 [Suppl 1]: 151–153

Harada S, Agarwal DP, Goedde H, Tagaki S, Ishikawa B (1982) Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. Lancet 2:827

Harada S, Misawa S, Agarwal DP, Goedde HW (1980) Liver alcohol dehydrogenase and aldehyde dehydrogenase in the Japanese: isoenzyme variation and its possible role in alcohol intoxication. Am J Hum Genet 32:8–15

Hsu LC, Bendel RE, Yoshida A (1988) Genomic structure of the human mitochondrial aldehyde dehydrogenase gene. Genomics 2:56–65

Hsu LC, Yoshida A, Mohandas T (1986) Chromosomal assignment of the genes for human aldehyde dehydrogenase-1 and aldehyde dehydrogenase-2. Am J Hum Genet 38:641–648

Klatskin C (1961) Alcohol and its relation to liver damage. Gastroenterology 41:443–451

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715–723

Kopun M, Propping P (1977) The kinetics of ethanol absorption and elimination in twins and supplementary repetitive experiments in singleton subjects. Eur J Clin Pharmacol 11:337–433

Lee S-C, Lin J-S, Chou F-J, Yin S-J (1989) Lung alcohol and aldehyde isoenzymes in Chinese. J Formosan Med Assoc 88:437–442

Madisen L, Hoar DI, Holroyd CD, Crisp M, Hodes ME (1987) DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. Am J Med Genet 27:379–390

Martin NG, Perl J, Oakeshott JG, Gibson JB, Starmer GA, Wilks AV (1985) A twin study of ethanol metabolism. Behav Genet 15:93–109

Mizoi Y, Ijiri I, Tatsuno Y, Kijima T, Fujiwara S, Adachi J (1979) Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. Pharmacol Biochem Behav 10:303–311

Mizoi Y, Kogame M, Fukunaga T, Ueno Y, Adachi J, Fujiwara S (1985) Polymorphism of aldehyde dehydrogenase and ethanol elimination. Alcohol 2:393–396

Ritchie JM (1980) The aliphatic alcohols. In: Gilman AG, Goodman LS, Gilman H (eds) Pharmacologic basis of therapeutics, 6th ed. Macmillian, New York, pp 376–390

Shibuya A, Yasunami M, Yoshida A (1989) Genotypes of alcohol dehydrogenase and aldehyde dehydrogenase loci in Japanese alcohol flushers and nonflushers. Hum Genet 82:14–16

Shibuya A, Yoshida A (1988) Genotypes of alcohol-metabolizing enzymes in Japanese with alcohol liver diseases: a strong association of the usual Caucasian-type aldehyde dehydrogenase gene ($ALDH_2^1$) with the disease. Am J Hum Genet 43:744–748

Smith M (1986) Genetics of human alcohol and aldehyde dehydrogenases. Adv Hum Genet 15:249–290

Sorensen TIA, Bentsen KD, Eghoje K, Orholm M, Hoybye G, Christoffersen P (1984) Prospective evaluation of alcohol abuse and alcoholic liver injury in men as predictors of development of cirrhosis. Lancet 2:241–244

Teng Y-S (1981) Human liver aldehyde dehydrogenase in Chinese and Asiatic Indians: gene deletion and its possible implication in alcohol metabolism. Biochem Genet 19: 107–114

Teng Y-S, Jehan S, Lie-Injo LE (1979) Human alcohol dehydrogenase ADH2 and ADH3 polymorphisms in ethnic Chinese and Indians of West Malaysia. Hum Genet 53: 87–90

Tsukahara M, Yoshida A (1989) Chromosomal assignment of the alcohol dehydrogenase cluster locus to human chromosome 4q21-23 by *in situ* hybridization. Genomics 4: 218–220

Tsukamoto S, Muto T, Nagoya T, Shimamura M, Saito M, Tainaka H (1989) Determinations of ethanol, acetaldehyde, and acetate in blood and urine during alcohol oxidation in man. Alcohol Alcohol 24:101–108

Wagner JG, Wilkinson PK, Sedman AJ, Kay DR, Weidler DJ (1976) Elimination of alcohol from human blood. J Pharm Sci 65:152–154

Wolff PH (1972) Ethnic differences in alcohol sensitivity. Science 175:449–450

Xu Y, Carr L, Bosron WF, Li T-K, Edenberg HJ (1988) Genotyping of human alcohol dehydrogenases at the ADH2 and ADH3 loci following DNA sequence amplification. Genomics 2:209–214

Yasunami MI, Kikuchi D, Sarapata D, Yoshida A (1990) The human class I alcohol dehydrogenase gene cluster: three genes are tandemly organized in an 80-kb-long segment of the genome. Genomics 7:152–158

Yoshida A, Huang I-Y, Ikawa M (1984) Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. Proc Natl Acad Sci USA 81:258–261

Yoshihara H, Sato N, Kamada T, Abe H (1983) Low Km ALDH isozyme and alcoholic liver injury. Pharmacol Biochem Behav 18 [Suppl 1]: 425–428