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Interaction between the Functional Polymorphisms of the Alcohol-Metabolism Genes in Protection against Alcoholism

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Summary

The genes that encode the major enzymes of alcohol metabolism, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), exhibit functional polymorphism. The variant alleles *ADH2*2* and *ADH3*1*, which encode high-activity ADH isoforms, and the *ALDH2*2* allele, which encodes the low-activity form of ALDH2, protect against alcoholism in East Asians. To investigate possible interactions among these protective genes, we genotyped 340 alcoholic and 545 control Han Chinese living in Taiwan at the *ADH2*, *ADH3*, and *ALDH2* loci. After the influence of *ALDH2*2* was controlled for, multiple logistic regression analysis indicated that allelic variation at *ADH3* exerts no significant effect on the risk of alcoholism. This can be accounted for by linkage disequlibrium between *ADH3*1* and *ADH2*2*. *ALDH2*2* homozygosity, regardless of the *ADH2* genotypes, was fully protective against alcoholism; no individual showing such homozygosity was found among the alcoholics. Logistic regression analyses of the remaining six combinatorial genotypes of the polymorphic *ADH2* and *ALDH2* loci indicated that individuals carrying one or two copies of *ADH2*2* and a single copy of *ALDH2*2* had the lowest risk (ORs 0.04–0.05) for alcoholism, as compared with the *ADH2*1/*1* and *ALDH2*1/*1* genotype. The disease risk associated with the *ADH2*2/*2-ALDH2*1/*1* genotype appeared to be about half of that associated with the *ADH2*1/*2-ALDH2*1/*1* genotype. The results suggest that protection afforded by the *ADH2*2* allele may be independent of that afforded by *ALDH2*2*.

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Introduction

Alcoholism is believed to be a multifactorial, polygenic disorder involving complex gene-with-gene and gene-with-environment interactions. Alcohol metabolism is one of the biological determinants that can significantly influence drinking behavior and the development of alcoholism (Yin 1994; Crabb et al. 1995). Most ethanol elimination occurs by oxidation to acetaldehyde and acetate, catalyzed principally by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (Edenberg and Bosron 1997). Both enzymes exhibit genetic polymorphism and ethnic variation (Smith 1986; Yoshida et al. 1991; Agarwal and Goedde 1992).

Four genes of the alcohol dehydrogenase family, coding for class I (*ADH1–3*) and class II (*ADH4*) enzymes, are significantly involved in the liver metabolism of ethanol (Han et al. 1998; Yin et al. 1999). Class I ADHs are homo-/heterodimeric enzymes containing α, β, or γ subunits with low (<5 mM) K_m values; class II $\pi\pi$ ADH has an intermediate K_m, 34 mM (Jörnvall and Höög 1995; Edenberg and Bosron 1997). Three allelic variants occur at the *ADH2* locus: *ADH2*1*, *ADH2*2*, and *ADH2*3*, which encode the subunits of β_1, β_2, and β_3, respectively. Two variants occur at the *ADH3* locus: *ADH3*1* and *ADH3*2*, which encode the subunits of γ_1 and γ_2, respectively. *ADH2*1* is the predominant allele among most world populations thus far studied (~90%), and *ADH2*2* is the predominant allele in East Asian populations (~70%). *ADH2*3* exists in populations of African origin (~20%) but appears to be very rare among the other ethnic groups. *ADH3*1* is the predominant allele among East Asians and Africans (~90%), whereas in whites it is about equally distributed with *ADH3*2* (Smith 1986; Agarwal and Goedde 1992). Both $\beta_2\beta_2$ and $\beta_3\beta_3$ ADHs exhibit 30–40-fold greater V_{max} for ethanol oxidation than $\beta_1\beta_1$, and the V_{max} for $\gamma_1\gamma_1$ is about twice that of $\gamma_2\gamma_2$ (Bosron et al. 1983; Yin et al. 1984; Burnell et al. 1989). The kinetic differences of the ADH allozymes can be attributed to a single amino-acid substitution (in β_2, his for arg-47 in

β_1; in β_3, cys for arg-369 in β_1; and in γ_1, arg for gln-271 in γ_2) that may affect dissociation of the coenzyme nicotinamide adenine dinucleotide (NADH), a rate-limiting step in catalysis (Eklund et al. 1987; Stone et al. 1993; Hurley et al. 1994). The second ile/val-349 exchange for γ_1/γ_2 appears not to alter enzyme activity, because it is located away from the active site (Eklund et al. 1987). The above functional polymorphisms are produced by single-nucleotide substitutions that occur in exon 3 of *ADH2*2*, exon 9 of *ADH2*3*, and exon 6 of *ADH3*2* (Smith 1986; Yoshida et al. 1991). The class I–ADH and class II–ADH genes have been mapped to the long arm of chromosome 4 in the region of 4q21-23 (Smith 1986; Yoshida et al. 1991). The class I–ADH genes cluster in a tandem array, spanning ~80 kb, in the order *5′-ADH3-ADH2-ADH1-3′* (Yasunami et al. 1990).

The human aldehyde dehydrogenase family is complex in a different way (Yoshida et al. 1998). The major forms responsible for oxidation of acetaldehyde in liver are low-K_m mitochondrial ALDH2 (0.20 μM) and cytosolic ALDH1 (33 μM) enzymes (Pietruszko 1983; Yin et al. 1995). There is a functional single-nucleotide polymorphism (SNP) occurring within exon 12 of the *ALDH2* gene, resulting in a glu/lys exchange at position 487 (Yoshida et al. 1991). About half of several East Asian populations carry the variant *ALDH2*2* allele, which is rarely seen in the other ethnic groups so far examined (Agarwal and Goedde 1992). Yoshida et al. (1983) had reported ALDH1 deficiency in an autopsy Japanese liver sample; however, this observation could not be substantiated in several studies using large number of surgical liver samples from the Japanese (n = 174; Takase et al. 1989) and Chinese (n = 142; Yin et al., unpublished data), suggesting that ALDH1 deficiency may be a rare mutation or, more likely, an artifact of processing of the postmortem liver. The ALDH1 deficiency has never been found in the livers of whites. The *ALDH1* and *ALDH2* genes have been mapped to chromosomes 9q21 and 12q24, respectively (Hsu et al. 1986; Yoshida et al. 1991). Recent reports have shown that the recombinant variant ALDH2 displayed a 260-fold increase in K_m for NAD^+ and an 11-fold reduction in V_{max} as compared with the normal enzymes (Farrés et al. 1994). The variant subunit also results in decreased activity of the tetrameric enzyme (Xiao et al. 1995; Wang et al. 1996), and accelerated degradation of the enzyme in transformed cell lines (Xiao et al. 1996). The X-ray structure of ALDH2 has revealed that the lys substitution for glu-487 may affect ion pairing with arg-475 from across the dimer interface, thereby indirectly diminishing the enzyme activity (Steinmetz et al. 1997). Therefore, the appearance of dominance, on starch gel electrophoresis, of the variant *ALDH2*2* in expression of enzyme activity (Crabb et al. 1989; Singh et al. 1989) can be explained by the activity of the homo- and heterotetrameric enzyme forms in the heterozygous *ALDH2*1/*2* liver samples being below the detection limit of gel staining. Subjects who are deficient in ALDH2 activity manifest elevated levels of acetaldehyde in blood, as well as facial flushing and tachycardia following the ingestion of an alcoholic beverage (Mizoi et al. 1979; Harada et al. 1981). This alcohol-induced sensitivity reaction is very similar to the aversive reaction caused by alcohol ingestion in patients being treated with the ALDH inhibitor disulfiram (Sellers et al. 1981).

It has been documented that the allele frequencies of *ADH2*2*, *ADH3*1*, and *ALDH2*2* are significantly decreased in alcoholics as compared with the general population of East Asians, including ethnic Han Chinese (Thomasson et al. 1991; Chen et al. 1996), Koreans (Shen et al. 1997), and Japanese (Higuchi 1994; Higuchi et al. 1994, 1995; Maezawa et al. 1995; Nakamura et al. 1996; Tanaka et al. 1997). It is worth noting that *ALDH2*2* homozygosity alone, regardless of the functional polymorphisms at *ADH2* and *ADH3*, appears to be completely protective against alcoholism, since no individual with this genotype has been found among the alcoholics (~10% of whom were of the heterozygous genotype; Higuchi et al. 1994). This full protection by *ALDH2*2/*2* homozygosity has been attributed to the low-dose alcohol sensitivity ascribable to the prolonged, large accumulation of acetaldehyde in blood (Peng et al. 1999). Association studies of functional polymorphisms involving the other alcohol-metabolizing enzymes, such as cytochrome P450 2E1 (*CYP2E1*), and alcoholism have thus far been negative (Iwahashi et al. 1995; Maezawa et al. 1995; Carr et al. 1996; Higuchi et al. 1996; Tanaka et al. 1997). Therefore, current molecular-genetic evidence supports the hypothesis proposed by Thomasson et al. (1991) that *ADH2*2*, *ADH3*1*, and *ALDH2*2* protect individuals from developing alcoholism through either faster production or slower removal of acetaldehyde, a metabolite that triggers aversive reactions.

This hypothesis also implies that the three alcohol-metabolism genes may act synergistically in ethanol metabolism to produce more acetaldehyde and, hence, more protection. To date, a systematic analysis of possible interactions among the allelic variations at the *ADH2*, *ADH3*, and *ALDH2* loci has been lacking. In fact, conflicting results exist in some preliminary and fragmentary observations. Higuchi et al. (1995) showed there was an interaction between the functional polymorphisms of *ALDH2* and *ADH2* in susceptibility to alcoholism among Japanese. Chen et al. (1996) reported that *ADH2*1*, *ADH3*2*, and *ALDH2*1* independently influenced the risk for alcoholism among Han Chinese. The latter argument has been refuted by the recent finding by Osier et al. (1999) that the observed frequency

differences of the functional polymorphism at *ADH3* in Han Chinese alcoholics and controls can be accounted for by the linkage disequilibrium with *ADH2*. Therefore, linkage disequilibrium between *ADH2* and *ADH3*, as well as dominance by the *ALDH2*2* variant (i.e., that homozygosity and heterozygosity for *ALDH2*2* may, respectively, fully or partially protect against alcoholism), should be taken into consideration for elucidating possible interactions between the alcohol-metabolism genes in relation to alcoholism. To solve this complex question, we have compared the haplotype frequencies of *ADH2* and *ADH3* with stratification of the *ALDH2* genotype in a total of 885 alcoholics and controls of Han Chinese descent in Taiwan and have performed multiple logistic regression to evaluate the relative risks of alcoholism in individuals carrying the combinatorial genotypes of *ALDH2*1/*1* or *ALDH2*1/*2* with one of the three different *ADH2* allelotypes.

Material and Methods

Two groups of Han Chinese subjects, alcohol-dependent according to the Diagnostic and Statistical Manual of Mental Disorder (DSM) III-R criteria (American Psychiatric Association 1987, pp 165–175), were patients from the Tri-Service General Hospital in Taipei (n = 150; 136 males and 14 females; mean ± SD age 39 ± 11 years; recruitment period 1994–1997) and the Taipei City Psychiatric Center (n = 141; 130 males and 11 females; mean ± SD age 40 ± 10 years; recruitment period 1994–1995), respectively. Han Chinese alcohol-dependent subjects from the Tri-Service General Hospital who were described in a previous report (Thomasson et al. 1991) were also included in the study, except that two subjects were deleted because of their lack of either the *ADH2* or the *ADH3* genotype, and that one subject with the *ADH2*1/*2*, *ADH3*1/*1*, and *ALDH2*1/*1* genotypes was added (n = 49; all males; mean ± SD age 40 ± 11 years; recruitment period 1989–1990). The diagnosis was carried out by attending psychiatrists at the two recruiting hospitals. The control Han Chinese subjects were male medical, dental, and pharmacy students at the National Defense Medical Center (n = 545; mean ± SD age 20 ± 2 years; recruitment period 1993–1996). Most of the participating students were nondrinkers; only some were occasional light drinkers, as revealed by a drinking-habit questionnaire. Of the 545 students, 105 were further assessed according to Mayfield et al. (1974); none of them responded positively to any of the questions regarding problem drinking. This study was approved by the Institutional Review Board for Human Studies; informed consent was obtained, and blood was drawn from each subject after the nature of the study had been explained.

Genomic DNA was extracted from leukocytes, as described elsewhere (Thomasson et al. 1991). Determination of the single-nucleotide polymorphic sites at exon 3 of the *ADH2* gene, exon 6 of the *ADH3* gene, and exon 12 of the *ALDH2* gene was carried out, as described elsewhere (Chao et al. 1994), by use of polymerase chain reaction (PCR)–directed mutagenesis and restriction-fragment-length polymorphism (RFLP). *ALDH2* genotypes were confirmed by a recent, improved PCR-RFLP method (Dandré et al. 1995).

Differences in genotypes and alleles for the separate polymorphisms were calculated by direct counting with the χ^2 test. The statistics program StatXact 4.0 (Cytel Software Cooperation) was used for correction of small sample sizes in some genotype groups of alcoholics. Functional polymorphisms of the *ADH* and *ALDH* genotypes for risk of alcoholism were evaluated by use of multiple logistic regression. In the *ALDH2* codominance model, the allelotypes for *ADH2*, *ADH3*, and *ALDH2* were denoted by three dummy variables (0, 1, and 2), and the homozygous usual type for each locus was chosen as the reference group. Since the *ALDH2*2* variant appeared dominant in expression of enzyme activity (Crabb et al. 1989; Xiao et al. 1996), two logistic-regression models were designed. In the complete-dominance model, *ALDH2*1/*2* and *ALDH2*2/*2* genotypes were combined into a single group. In the partial-dominance model, codings for *ALDH2*1/*1*, *ALDH2*1/*2*, and *ALDH2*2/*2* were set as continuous variables to 1.0, 0.2, and 0, respectively, since the specific activities of ALDH2 in surgical liver samples with the *ALDH2*1/*2* and *ALDH2*2/*2* genotypes were ~0.2 and ~0, respectively, relative to the specific activity of the *ALDH2*1/*1* livers (Yin et al., unpublished data). Six combinations of the *ADH2* and *ALDH2* genotypes were evaluated for risk of the development of alcoholism by logistic-regression analysis (three combinations with the homozygous *ALDH2*2/*2* genotype were excluded, since not a single alcoholic subject with this genotype was found). The χ^2 test and logistic-regression analysis were performed with the SPSS for Windows statistics program (release 8.0.0).

Haplotype frequencies and linkage-disequilibrium coefficients of the *ADH2* and *ADH3* genes were estimated by use of the ARLEQUIN program (1997) kindly provided by Schneider et al. The haplotype method developed by Valdes and Thomson (1997) was used to evaluate the relative importance of the two polymorphic sites in determining susceptibility to alcoholism.

Results

Genotype and allele distributions of *ADH2*, *ADH3*, and *ALDH2* were found not to be significantly different among the three subgroups of Han Chinese alcoholics recruited from the two hospitals (table 1), one of which

Table 1

Genotype and Allele Distributions of *ADH* and *ALDH2*

Gene and Group[a]	Subject Number	Genotype Number (Frequency)[b]				Allele Number (Frequency)		
		*1/*1	*1/*2	*2/*2	*P* Value[c]	*1	*2	*P* Value[c]
ADH2:								
Controls	545	43 (0.08)	205 (0.38)	297 (0.54)		291 (0.27)	799 (0.73)	
Alcoholics:								
TSGH1	49	18 (0.37)	15 (0.31)	16 (0.33)	$<10^{-6}$	51 (0.52)	47 (0.48)	$<10^{-6}$
TSGH2	150	50 (0.33)	47 (0.31)	53 (0.35)	$<10^{-6}$	147 (0.49)	153 (0.51)	$<10^{-6}$
TCPC	141	62 (0.44)	44 (0.31)	35 (0.25)	$<10^{-6}$	168 (0.60)	114 (0.40)	$<10^{-6}$
Total	340	130 (0.38)	106 (0.31)	104 (0.31)	$<10^{-6}$	366 (0.54)	314 (0.46)	$<10^{-6}$
ADH3:								
Controls	545	448 (0.82)	93 (0.17)	4 (0.01)		989 (0.91)	101 (0.09)	
Alcoholics:								
TSGH1	49	30 (0.61)	16 (0.33)	3 (0.06)	1.3×10^{-3}	76 (0.78)	22 (0.22)	2×10^{-4}
TSGH2	150	111 (0.74)	32 (0.21)	7 (0.05)	1.4×10^{-3}	254 (0.85)	46 (0.15)	3.1×10^{-3}
TCPC	141	97 (0.69)	37 (0.26)	7 (0.05)	1×10^{-5}	231 (0.82)	51 (0.18)	$<10^{-6}$
Total	340	238 (0.70)	85 (0.25)	17 (0.05)	$<10^{-6}$	561 (0.82)	119 (0.18)	$<10^{-6}$
ALDH2:								
Controls	545	304 (0.56)	218 (0.40)	23 (0.04)		826 (0.76)	264 (0.24)	
Alcoholics:								
TSGH1	49	44 (0.90)	5 (0.10)	0	$<10^{-6}$	93 (0.95)	5 (0.05)	$<10^{-6}$
TSGH2	150	123 (0.82)	27 (0.18)	0	$<10^{-6}$	273 (0.91)	27 (0.09)	$<10^{-6}$
TCPC	141	116 (0.82)	25 (0.18)	0	$<10^{-6}$	257 (0.91)	25 (0.09)	$<10^{-6}$
Total	340	283 (0.83)	57 (0.17)	0	$<10^{-6}$	623 (0.92)	57 (0.08)	$<10^{-6}$

[a] Alcoholic patients were recruited from the Tri-Service General Hospital, Taipei (TSGH1 and TSGH2) and the Taipei City Psychiatric Center (TCPC). Data for TSGH1 alcoholics were from Thomasson et al. (1991). No significant difference was found in the distribution of the genotype numbers of *ADH2*, *ADH3*, and *ALDH2* among the TSGH1, TSGH2, and TCPC alcoholics ($P = .30–.54$).

[b] Because they are rounded to two significant figures, frequencies may not sum to 1.00.

[c] To increase statistical power, genotype number and allele number, instead of the frequency, were used in comparison.

was the recruitment site of a smaller study reported on elsewhere (Thomasson et al. 1991). The alcoholic subgroups and combined group were significantly different from the controls in allelic variations at the separate alcohol-metabolism–gene loci (table 1). Both male alcoholics ($n = 315$) and female alcoholics ($n = 25$) exhibited significant differences, as compared with male controls, in the distributions of the genotypes and alleles of *ADH2*, *ADH3*, and *ALDH2* ($P \leqslant .006$; data not shown). No significant difference was found between the male and female alcoholic groups, except for a marginal difference ($P = .048$) in the allele number of *ADH2*. This is probably due to the small size of the female group. The allele frequencies of *ADH2*1* (.27) and *ADH2*2* (.73) were coincidentally close to those of *ALDH2*2* (.24) and *ALDH2*1* (.76) in Han Chinese controls. These frequencies are similar to those reported for the Japanese population (Higuchi et al. 1995). It is worth noting that results of the comparison between alcoholics and controls shown in table 1, as well as in subsequent analyses, are likely to be conservative estimates, because students were chosen as controls. Alcoholism is usually diagnosed later in life in Taiwan (Helzer et al. 1990), and, therefore, the power to determine differences should be somewhat reduced, since susceptibility in some younger controls may be revealed later in life. This possible reduction in power can be minimized by employing a large sample number, as we did in the present study, in view of the low lifetime prevalence rate of male alcohol dependence in Taiwan, ~3% (Helzer et al. 1990).

To determine whether the effects of the *ADH2* and *ADH3* genotypes were independent of the *ALDH2* genotype, we compared the subgroups containing individuals homozygous for the *ALDH2*1* allele (table 2). All these individuals were predicted to have normal ALDH2 activity. Among these subjects, the differences between alcoholics and controls in the numbers of *ADH2*2* and *ADH3*1* alleles remained highly significant. This is in agreement with previous observations, made with smaller sample sizes, in Han Chinese and Japanese alcoholics (Thomasson et al. 1991; Nakamura et al. 1996). To further evaluate the separate roles of the functional polymorphisms of the *ADH2*, *ADH3*, and *ALDH2* in predisposition to alcoholism, logistic regression analysis was performed, assuming different dominance models for *ALDH2* (table 3). Since none of the interaction parameters were statistically significant in the three logistic models, only the main effects of the sep-

Table 2

Genotype and Allele Distribution of *ADH2* and *ADH3* among Individuals Homozygous for *ALDH2*1*

Gene and Group	Subject Number	Genotype Number (Frequency)[a]				Allele Number (Frequency)		
		*1/*1	*1/*2	*2/*2	*P* Value	*1	*2	*P* Value
ADH2:								
Controls	304	26 (0.09)[b]	109 (0.36)[b]	169 (0.56)[b]		161 (0.26)[b]	447 (0.74)[b]	
Alcoholics	283	108 (0.38)[c]	89 (0.32)[c]	86 (0.30)[c]	$<10^{-6}$	305 (0.54)[c]	261 (0.46)[c]	$<10^{-6}$
ADH3:								
Controls	304	249 (0.82)[b]	51 (0.17)[b]	4 (0.01)[b]		549 (0.90)[b]	59 (0.10)[b]	
Alcoholics	283	198 (0.70)[c]	73 (0.26)[c]	12 (0.04)[c]	1.5×10^{-3}	469 (0.83)[c]	97 (0.17)[c]	3×10^{-4}

[a] Because they are rounded to two significant figures, frequencies may not sum to 1.00.

[b] Controls homozygous for *ALDH2*1* were not significantly different from controls who have an *ALDH2*2* allele (P = .30–.70), refer to table 1.

[c] No significant difference was found in the distribution of the genotype number and allele number, either for *ADH2* or for *ADH3* genes among the component TSGH1, TSGH2, and TCPC alcoholics (P = .24–.80), refer to table 1.

arate genes were analyzed. The codominance model was based on the assumption that the activity of the mixture of ALDH2E/K homo- and heterotetramers forming from random combination of the E and K subunits represents the mean value of the activities of ALDH2E (the usual-type enzyme with glutamic acid at position 487) and ALDH2K (the variant type containing lys-487). The complete-dominance model assumed both the homotetrameric ALDH2K and the heterotetrameric ALDH2K/E enzymes to be inactive. The partial-dominance model assumed the homotetrameric variant enzyme to be inactive but assumed the heterotetrameric enzymes to have some residual activity. As the influences of the *ADH2* and *ALDH2* genes were adjusted, allelic variations at the *ADH3* locus failed in all three of the tested models to show a significant effect (P = .23–.56) on risk for alcoholism. By contrast, the *ADH2*1/*2* and *ADH2*2/*2* genotypes exhibited highly significant protection (odds ratios, .12–.19; 95% confidence intervals, .07–.30) as compared with the *ADH2*1/*1* genotype. The effect of the *ADH2* gene seemed to be independent of that for *ALDH2*, since the odds ratios remained nearly unchanged with the different models of *ALDH2* dominance. The power of the homozygous *ALDH2*2/*2* genotype for protection against alcoholism did not reach significance (P = .36) in the codominance model, apparently because no individual with that genotype was found among alcoholics.

Linkage disequilibrium and relative haplotype frequencies of *ADH2* and *ADH3* in alcoholics and controls were estimated and were evaluated to discern whether an influence of the polymorphic *ADH3* gene on susceptibility to alcoholism is nullified by *ADH2*. The *ALDH2* genotypes were stratified to eliminate possible confounding by the variant *ALDH2*2* in assessing association of the haplotypes with alcoholism. Both alcoholics and controls exhibited significant linkage disequilibrium of *ADH2* and *ADH3* ($P \leqslant .013$) (table 4). In comparing the haplotype frequencies of *ADH2* and *ADH3* in alcoholics and in controls (table 5), significant differences ($P \leqslant 4.4 \times 10^{-5}$) were found in the *ALDH2*1/*1* genotype with respect to allelic variations at *ADH2* when the *ADH3* polymorphism was controlled for. This difference was not found in a previous study that controlled for the *ADH3*2* allele, but the sample size of that study was small (Osier et al. 1999). As shown in table 5, when the *ADH2* polymorphism was controlled for, allelic variations at *ADH3* did not show significant difference (P = .06–.66) between alcoholics and controls, suggesting that the observed effect of *ADH3* (tables 1 and 2) is due to the linkage disequilibrium of the *ADH2* and *ADH3* alleles (table 5).

To evaluate the interactions between the functional polymorphisms at *ADH2* and *ALDH2* in relation to alcoholism, six combinatorial genotypes of those loci were analyzed by logistic regression (table 6). Three other genotype combinations containing the homozygous *ALDH2*2/*2* were excluded, because of the absence of individuals with that genotype in the alcoholic group. As compared with those having both *ADH2*1/*1* and *ALDH2*1/*1*, subjects carrying the remaining five combinatorial genotypes exhibited varying degrees of protection against alcoholism, with the *ADH2*2/*2-ALDH2*1/*2* individuals having the least risk for developing the disease (odds ratio 0.04) and the *ADH2*1/*1-ALDH2*1/*2* individuals having the greatest risk (odds ratio 0.33).

Discussion

To date, *ADH* and *ALDH* are the only so-called alcoholism genes which have been firmly established to influence vulnerability to the disease. One of the major reasons is that both the genotypes and the phenotypes

Table 3

Multiple Logistic Regression Analysis of the Functional Polymorphisms of *ADH2*, *ADH3*, and *ALDH2* for Risk of Alcoholism[a]

Model of *ALDH2* Dominance and Variable[b]	Regression Coefficient	Standard Error	*P* Value	Odds Ratio	95% Confidence Interval
Codominance[c]:					
ADH2:					
*ADH2*1/*2*	−1.68	.23	$<10^{-6}$	.19	.12–.30
*ADH2*2/*2*	−2.11	.24	$<10^{-6}$	.12	.08–.19
ADH3:					
*ADH3*1/*2*	−.12	.21	.56	.88	.59–1.33
*ADH3*2/*2*	.72	.62	.25	2.05	.60–6.97
ALDH2:					
*ALDH2*1/*2*	−1.26	.18	$<10^{-6}$	.28	.20–.40
*ALDH2*2/*2*	−6.89	7.52	.36	.001	0–2544
Constant	1.46	.22	$<10^{-6}$		
Complete dominance[d]:					
ADH2:					
*ADH2*1/*2*	−1.69	.23	$<10^{-6}$	.18	.12–.29
*ADH2*2/*2*	−2.13	.24	$<10^{-6}$	.12	.07–.19
ADH3:					
*ADH3*1/*2*	−.12	.21	.56	.89	.59–1.33
*ADH3*2/*2*	.74	.63	.24	2.10	.62–7.19
ALDH2	−1.36	.18	$<10^{-6}$	.26	.18–.37
Constant	1.47	.22	$<10^{-6}$		
Partial dominance[e]:					
ADH2:					
*ADH2*1/*2*	−1.69	.23	$<10^{-6}$	.18	.11–.29
*ADH2*2/*2*	−2.12	.24	$<10^{-6}$	.12	.07–.19
ADH3:					
*ADH3*1/*2*	−.12	.21	.55	.88	.59–1.33
*ADH3*2/*2*	.74	.63	.23	2.10	.61–7.16
ALDH2	1.70	.22	$<10^{-6}$	5.44	3.51–8.45
Constant	−.22	.27	.42		

[a] Alcoholics, $n = 340$; controls, $n = 545$.

[b] Codings of the genotypes of *ADH2* and *ADH3* are **1/*1* = 0, **1/*2* = 1, and **2/*2* = 2. Reference groups for the *ADH2* and *ADH3* genotypes are *ADH2*1/*1* and *ADH3*1/*1*, respectively.

[c] Codings of *ALDH2* genotypes are **1/*1* = 0, **1/*2* = 1, and **2/*2* = 2.

[d] Codings of *ALDH2* genotypes are **1/*1* = 0, **1/*2* and **2/*2* = 1, assuming *ALDH2*2* is a completely dominant allele over activity loss of the heterotetrameric enzymes.

[e] Coding values of *ALDH2* genotypes are **1/*1* = 1.0, **1/*2* = 0.2 and **2/*2* = 0, assuming relative activities of ALDH2 in the *ALDH2*1/*1*, *ALDH2*1/*2*, and *ALDH2*2/*2* livers are 1.0, 0.2 and 0, respectively. In this model, codings of *ALDH2* genotypes represent continuous variables in reflection of relative enzyme activity rather than categorical codings used in the codominance and complete dominance models.

of allelic variations at these loci have been well defined. Complex interrelationships between functional polymorphisms of the alcohol-metabolism genes shown in this study partly illustrate the current concept that alcoholism is a complex behavioral trait that is influenced by multiple genes as well as by sociocultural factors (Cloninger 1987; Goldman 1993).

Alcohol-drinking behavior can be affected by rewarding or aversive effects of alcohol on the brain and the body. Acetaldehyde, high concentrations of which in the body are produced by an inborn error of ethanol metabolism, deters the individuals from excessive drinking on account of unpleasant cardiovascular effects and subjective symptoms, thereby reducing the risk of developing alcoholism (Mizoi et al. 1979; Harada et al. 1981; Peng et al. 1999). This explains at least part of the reason why, in combination with the other possible biological and environmental factors, Han Chinese (among whom the allele frequency of *ALDH2*2* is 0.24; see table 1) living in Taiwan exhibited an ~8-fold lower lifetime prevalence of alcohol dependence than did the Atayal natives (among whom the allele frequency of *ALDH2*2* is 0.05; Hwu et al. 1990; Thomasson et al. 1994). Importantly, a single biological determinant, *ALDH2*2/*2* homozygosity, appeared sufficient to completely protect against the development of alcoholism (table 1; Hig-

Table 4

Linkage Disequilibrium Analysis of *ADH2* and *ADH3* with Stratification of *ALDH2* Genotypes

ALDH2 Genotype and Group	Haplotype Number (2*N*)	Haplotype Frequency of *ADH2* and *ADH3*[a]				D[b]	D'[c] (*P* Value)
		*3*1–2*1*	*3*1–2*2*	*3*2–2*1*	*3*2–2*2*		
*ALDH2*1/*1:*							
Controls	608	.192 (.016)	.711 (.018)	.072 (.011)	.025 (.006)	.047	.654 ($<10^{-6}$)
Alcoholics	566	.376 (.020)	.452 (.021)	.164 (.016)	.007 (.004)	.072	.910 ($<10^{-6}$)
*ALDH2*1/*2:*							
Controls	436	.209 (.019)	.697 (.022)	.071 (.012)	.023 (.007)	.045	.661 ($<10^{-6}$)
Alcoholics	114	.386 (.046)	.421 (.046)	.149 (.034)	.044 (.019)	.046	.511 (.013)
Total:							
Controls	1090	.197 (.012)	.710 (.014)	.070 (.008)	.023 (.005)	.045	.662 ($<10^{-6}$)
Alcoholics	680	.377 (.019)	.447 (.019)	.162 (.014)	.015 (.005)	.067	.820 ($<10^{-6}$)

[a] Haplotype frequencies were estimated using the ARLEQUIN program (1997). The haplotypes are labeled as the sites occur in order from 5′ to 3′ in the class I *ADH* cluster: *ADH3*, functional alleles 1 or 2 in exon 6; *ADH2*, functional alleles 1 or 2 in exon 3; e.g. *3*1–2*1* represents haplotype of *ADH3*1* and *ADH2*1*. Values are mean ± standard deviation (in parenthesis). Some groups' frequencies do not sum to 1,000 due to rounding errors.

[b] Linkage disequilibrium coefficient.

[c] Standardized linkage disequilibrium coefficient.

uchi et al. 1994). This can be attributed to the total loss of ALDH2 activity, resulting from two copies of the missense mutation, which causes the subjects either to abstain or to deliberately moderate alcohol consumption because of prior experience of an unpleasant reaction following drinking (Peng et al. 1999). The *ALDH2*1/*2* heterozygosity displays partial protection, since it was found in only 10%–18% of Han Chinese alcoholics (versus 40% of controls; see table 1). Interestingly, the frequency of the heterozygotes in the alcoholic population seemed to be rising in the period between 1989 and 1997. A similar observation was noticed among the Japanese alcoholics by Higuchi et al. (1994). These findings indicate that among *ALDH2*1/*2* alcoholics, the other biological determinants, such as functional polymorphism of the *ADH* genes, as well as sociocultural factors, are contributing increasingly to development of the disease. Both complete dominance (Crabb et al. 1989; Singh et al. 1989) and partial dominance (Xiao et al. 1995, 1996; Wang et al. 1996) of the variant ALDH2K subunit over activity loss of the tetrameric enzymes have been described. Surgical liver samples with the *ALDH2*1/*2* genotype exhibited ~20% of the specific activity in the *ALDH2*1/*1* liver samples, as measured with 3 μM acetaldehyde, whereas the specific activity in the *ALDH2*2/*2* livers was undetectable (Yin et al., unpublished data). These levels of specific activity are close to those predicted from a model study using transduced cell lines (Xiao et al. 1996). The partial-dominance model has been substantiated by the strikingly different blood acetaldehyde profiles found in subjects with the different *ALDH2* allelotypes, but carrying the identical *ADH2* and *ADH3* genotypes, following a low dose of alcohol (Peng et al. 1999). It seems clear that the mitochondrial ALDH2 and the cytosolic ALDH1 are mainly responsible for oxidation of acetaldehyde in the homozygous *ALDH2*1/*1* and *ALDH2*2/*2* individuals, respectively, and that the residual ALDH2 activity, plus that of ALDH1, contributes to removal of acetaldehyde in the heterozygotes during alcohol consumption.

Involvement of the functional polymorphisms at the *ADH3* locus in susceptibility to alcoholism has been an intriguing and controversial issue. In theory, white subjects would be best for testing this hypothesis; the power to detect differences between genotypes is greater in whites, because of nearly homogeneous distribution of *ADH3*1* and *ADH3*2*, nearly equal distribution of *ADH2*1*, and the absence of the confounding effects of *ALDH2*2*. Results of studies of association between the *ADH3* allelic variations and alcoholism in various European populations have so far been negative (Couzigou et al. 1990; Gilder et al. 1993; Parés et al. 1994). This implies that the effect of *ADH3* polymorphisms on propensity to alcoholism is neutral or very small. However, a positive association when the *ALDH2* genotype is controlled for has been found consistently among East Asians, including the Han Chinese (table 2; Thomasson et al. 1991; Chen et al. 1996) and Japanese (Nakamura et al. 1996). These contradictory findings have been clarified by the multiple logistic regression analysis of *ADH3*, *ADH2,* and *ALDH2* shown in table 3. As the *ADH2* genotype was adjusted, allelic variations at *ADH3* did not exhibit a significant effect on risk of alcoholism, irrespective of which model of *ALDH2* dominance was applied. This is in agreement with the negative finding among white alcoholics (Couzigou et al. 1990; Gilder et al. 1993; Parés et al. 1994). Nullification of the influence of *ADH3* on the susceptibility of East Asians to alcoholism can be fully ascribed to the

Table 5

Relative Haplotype Frequencies of *ADH2* and *ADH3* in Han Chinese Alcoholics and Controls

ALDH2 Genotype	Ratio of Haplotype Frequency		*P* Value[a]
	Alcoholics	Controls	
*ALDH2*1/*1:*	*2N* = 566	*2N* = 608	
$\frac{ADH3*1-2*1}{ADH3*1-2*2}$	$\frac{0.376}{0.452}$ = 0.832	$\frac{0.192}{0.711}$ = 0.270	$<10^{-6}$
$\frac{ADH3*2-2*1}{ADH3*2-2*2}$	$\frac{0.164}{0.007}$ = 23.4	$\frac{0.072}{0.025}$ = 2.88	4.4×10^{-5}
$\frac{ADH3*1-2*1}{ADH3*2-2*1}$	$\frac{0.376}{0.164}$ = 2.29	$\frac{0.192}{0.072}$ = 2.67	.44
$\frac{ADH3*1-2*2}{ADH3*2-2*2}$	$\frac{0.452}{0.007}$ = 64.6	$\frac{0.711}{0.025}$ = 28.4	.12
*ALDH2*1/*2:*	*2N* = 114	*2N* = 436	
$\frac{ADH3*1-2*1}{ADH3*1-2*2}$	$\frac{0.386}{0.421}$ = 0.917	$\frac{0.209}{0.697}$ = 0.300	.018
$\frac{ADH3*2-2*1}{ADH3*2-2*2}$	$\frac{0.149}{0.044}$ = 3.39	$\frac{0.071}{0.023}$ = 3.09	.76
$\frac{ADH3*1-2*1}{ADH3*2-2*1}$	$\frac{0.386}{0.149}$ = 2.59	$\frac{0.209}{0.071}$ = 2.94	.66
$\frac{ADH3*1-2*2}{ADH3*2-2*2}$	$\frac{0.421}{0.044}$ = 9.57	$\frac{0.697}{0.023}$ = 30.3	.06
Total:	*2N* = 680	*2N* = 1090	
$\frac{ADH3*1-2*1}{ADH3*1-2*2}$	$\frac{0.377}{0.447}$ = 0.843	$\frac{0.197}{0.710}$ = 0.277	$<10^{-6}$
$\frac{ADH3*2-2*1}{ADH3*2-2*2}$	$\frac{0.162}{0.015}$ = 10.8	$\frac{0.070}{0.023}$ = 3.04	8×10^{-4}
$\frac{ADH3*1-2*1}{ADH3*2-2*1}$	$\frac{0.377}{0.162}$ = 2.33	$\frac{0.197}{0.070}$ = 2.81	.25
$\frac{ADH3*1-2*2}{ADH3*2-2*2}$	$\frac{0.447}{0.015}$ = 29.8	$\frac{0.710}{0.023}$ = 30.9	.31

[a] Significance was measured using the 2 × 2 contingency table of Valdes and Thomson (1997). The method tests the null hypothesis that, in a subgroup of the samples with a specific allele at one site, the ratio of the number of chromosomes with the two alleles at the second site is the same in alcoholics and controls.

existence of linkage disequilibrium between *ADH3* and *ADH2* (tables 4 and 5). The observed significant reduction in the frequency of *ADH3*1* in alcoholics as compared to controls (table 2) is caused by its linkage to *ADH2*2* (table 5). The *ADH2* allelic variation is sufficient to explain the different levels of susceptibility to alcoholism. Therefore, our results have confirmed and expanded the previous finding by Osier et al. (1999). In view of the close vicinity of the *ADH2* and *ADH3* loci, which are only ~15 kb apart (Yasunami et al. 1990), it is reasonable to see linkage disequilibrium between the functional and neutral polymorphisms of the two loci among various populations (table 4; Edman and Maret 1992; Higuchi 1994; Chen et al. 1996, 1997; Osier et al. 1999). Possible explanations for the lack of influence of *ADH3* on susceptibility to alcoholism include: (a) a much smaller difference in V_{max} values for ethanol oxidation of the $\gamma\gamma$ allozymes compared with $\beta\beta$ allozymes (Bosron et al. 1983; Yin et al. 1984), (b) low expression of the γ subunits in liver (~20% that of the β subunits in terms of protein content; Yin et al., unpublished data), and (c) large individual variation in alcohol elimination (Kalant 1996).

Interactions between the functional polymorphisms of *ADH2* and *ALDH2* have been evaluated by logistic regression of the six combinatorial genotypes between alcoholics and controls (table 6). As the *ALDH2*1/*1* genotype is controlled for, subjects with the heterozygous *ADH2*1/*2* and homozygous *ADH2*2/*2* had 5.3- and 8.3-fold less risk, respectively, for alcoholism than did the usual-type *ADH2*1/*1* individuals. Since the odds ratio of the *ADH2*2/*2-ALDH2*1/*1* genotype was about half that of the *ADH2*1/*2-ALDH2*1/*1* genotype, the effect of the *ADH2*2* allele on risk of alcoholism seems to be additive in the *ALDH2*1/*1* individuals. When the *ADH2*1/*1* genotype is controlled for, *ALDH2*1/*2* is shown to confer ~3-fold less risk than *ALDH2*1/*1*. In view of the complete protection against alcoholism by *ALDH2*2/*2*, it is interesting to find that individuals with the combinatorial *ADH2*1/*1-ALDH2*1/*2* genotype had 1.7-fold greater risk than those with *ADH2*1/*2-ALDH2*1/*1* (table 6). To validate the comparison of the relative risks of alcoholism in these two genotypes, multiple logistic regression of the six combinatorial genotypes has been further analyzed with *ADH2*1/*2-ALDH2*1/*1*, instead of *ADH2*1/*1-ALDH2*1/*1*, as reference group. The *ADH2*1/*1-ALDH2*1/*2* genotype was found not to be significantly different from the *ADH2*1/*2-ALDH2*1/*1* genotype ($P = .145$), whereas the remaining combinatorial genotypes still showed a significant difference ($P \leqslant .013$) from the reference genotype (data not shown). The subjects who were carrying *ADH2*1/*2-ALDH2*1/*2* and *ADH2*2/*2-ALDH2*1/*2* had 20- and 25-fold less risk for alcoholism, respectively, than did those carrying *ADH2*1/*1-ALDH2*1/*1* (table 6). This would suggest that the combination of the functional variants at both *ADH2* and *ALDH2* confers 2–7-fold less risk than do the three other combinatorial genotypes that include either *ADH2*1/*1* or *ALDH2*1/*1*. To evaluate the relative influence on risk of alcoholism of *ADH2*2* and *ALDH2*2*, further investigations, such as family studies assessing the relative penetrance of the variant alleles, are required. However, the effects of *ADH2*2* and *ALDH2*2* on risk of alcoholism seem to be independent of each other. This conclusion is based on the following findings: (a) the odds ratios of the genotypes *ADH2*1/*2* and *ADH2*2/*2* remained nearly unchanged, regardless of the models of *ALDH2* dominance applied (table 3); (b) the odds ratios of the combinatorial genotypes *ADH2*1/*2-ALDH2*1/*2* and *ADH2*2/*2-ALDH2*1/*2* appeared to be close to the calculated product of the odds ratios of the component genotypes (see table 6; for *ADH2*1/*2-ALDH2*1/*1* and *ADH2*1/*1-ALDH2*1/*2*, $0.19 \times 0.33 = 0.06$ and for

Table 6

Logistic Regression Analysis of Combinatorial Genotypes of *ADH2* and *ALDH2* for Risk of Alcoholism

Variable[a]	Regression Coefficient	Standard Error	*P* Value	Odds Ratio	95% Confidence Interval
*ADH2*1/*2 – ALDH2*1/*1*	−1.64	.26	$<10^{-6}$	.19	.12–.32
*ADH2*2/*2 – ALDH2*1/*1*	−2.12	.26	$<10^{-6}$	.12	.07–.20
*ADH2*1/*1 – ALDH2*1/*2*	−1.11	.39	.0047	.33	.15–.71
*ADH2*1/*2 – ALDH2*1/*2*	−3.08	.34	$<10^{-6}$	.05	.02–.09
*ADH2*2/*2 – ALDH2*1/*2*	−3.27	.33	$<10^{-6}$	.04	.02–.07
Constant	1.43	.22	$<10^{-6}$		

[a] Alcoholics, $n = 340$; controls, $n = 522$. *ALDH2*2/*2* genotype was not included for comparison since no single such individual was found in alcoholic group. Reference group is *ADH2*1/*1 – ALDH2*1/*1*.

*ADH2*2/*2-ALDH2*1/*1* and *ADH2*1/*1-ALDH2*1/*2*, 0.12 × 0.33 = 0.04); (c) the odds ratios and 95% confidence intervals for *ADH2*1/*2* and *ADH2*2/*2* obtained for (a) and (b) above were almost identical; (d) the magnitudes of the reduction in the haplotype frequency of *ADH3*1-2*2* and of the increase in the haplotype frequency of *ADH3*1-2*1* among the alcoholics versus the controls remained virtually unchanged, irrespective of the *ALDH2* genotypes (table 5; for *ALDH2*1/*1*, 0.452 ÷ 0.711 = 0.64 and 0.376 ÷ 0.192 = 2.0, respectively, and for *ALDH2*1/*2*, 0.421 ÷ 0.697 = 0.60 and 0.386 ÷ 0.209 = 1.8, respectively). Independent effects of the functional polymorphisms of *ADH2* and *ALDH2* on alcoholism would imply that the molecular protection mechanism of *ADH2* may not be mainly through the pathway of blood acetaldehyde accumulation after alcohol ingestion, as has been firmly established with *ALDH2* (Mizoi et al. 1979; Harada et al. 1981; Peng et al. 1999). Indeed, during alcohol consumption allelic variations at *ADH2* did not cause significant elevation of blood acetaldehyde levels, which were actually near zero, in the homozygous *ALDH2*1/*1* Japanese (at a dose of 0.4 g/kg ethanol; Mizoi et al. 1994) and Han Chinese (at a dose of 0.5 g/kg ethanol, Yin et al., unpublished data). The rates of elimination from blood at saturating ethanol concentrations for class I ADHs also did not show a significant difference among the three *ADH2* genotypes (Mizoi et al. 1994). The alcohol-induced facial flushing appeared to be associated solely with the *ALDH2* polymorphism, and not with *ADH2*, following a low dose of 0.3 g/kg ethanol (Yin et al., unpublished data). Therefore, the recent new findings discussed herein seem not to be supportive of the long-standing hypothesis that *ADH2*2*, which encodes the high-activity β_2 subunits, produces facial flushing (Stamatoyannopoulos et al. 1975) and other dysphoric reactions through the accumulation of acetaldehyde in blood, thereby influencing drinking behavior (Thomasson et al. 1991).

Nakamura et al. (1996) proposed that the *ADH2*1* allele may play a special role in the etiology of alcoholism in heterozygous *ALDH2*1/*2* alcoholics. This was based on the observation that Japanese alcoholics with *ALDH2*1/*2* showed a 2.4-fold higher frequency of *ADH2*1/*1* than alcoholics with *ALDH2*1/*1* (total alcoholics, $n = 53$). In the present study, however, alcoholics homozygous for *ADH2*1* with *ALDH2*1/*1* and *ALDH2*1/*2* showed very similar frequencies: 0.382 (108/283) and 0.386 (22/57), respectively. In fact, the haplotype frequencies of *ADH3*1-2*1* in the alcoholics carrying *ALDH2*1/*1* and *ALDH2*1/*2* are also similar: 0.376 and 0.386, respectively (table 5). Therefore, polymorphic *ADH2* and *ALDH2* appear not to have a special interaction. Higuchi et al. (1995) found that for Japanese alcoholics heterozygous for *ALDH2*1/*2* the odds ratio of *ADH2*1/*1* was significantly high (2.1), whereas those of *ADH2*2/*2* and *ADH2*1/*2* were ~0.1–0.3, suggesting that homozygous *ADH2*1/*1* may overcome the protective effect of heterozygous *ALDH2*1/*2* in predisposition to alcoholism. This result, however, was derived from an incorrect method of data analysis. In their χ^2 test, no fixed reference group was used in evaluation of the relative risk; that is, all the non-test genotype groups were combined as the reference in multiple comparisons (Higuchi et al. 1995). Therefore, the observed significant effect on risk for alcoholism of the interaction of *ADH2*1/*1* and *ALDH2*1/*2* is, in fact, nonexistent. Chen et al. (1996) recently reported that the functional polymorphisms of *ADH2*, *ADH3*, and *ALDH2* independently influenced the disease risk among Han Chinese alcoholics. Again, the conclusion was drawn from an incorrect multiple logistic regression using continuous-variable instead of dummy-variable codings with an insufficient number of alcoholics ($n = 46$).

Association between reduced alcohol consumption or reduced risk of alcoholism and the variant *ADH2*2* allele has recently been found in other ethnic groups that predominantly carry *ALDH2*1/*1*, including Australians of European descent (Whitfield et al. 1998), Jews in Israel (Neumark et al. 1998), Mongolians in China (Shen et al. 1997), and Atayal natives of Taiwan (Tho-

masson et al. 1994). This is consistent with the findings in this study that *ADH2* may affect vulnerability to alcoholism independent of *ALDH2*. The molecular mechanism of the *ADH2* effect remains unclear. There are a few possible explanations. (a) Target organs of class I ADH other than the liver may be involved, such as the brain (Zimatkin and Deitrich 1997) and the heart, in which only $\beta\beta$ allozymes are expressed (Yin 1994). (b) Target substrates of class I ADH may be unrelated to the conventional ethanol/acetaldehyde; for instance, alcohol/aldehyde metabolites of the neurotransmitter dopamine (Mårdh and Vallee 1986), serotonin (Consalvi et al. 1986; Svensson et al. 1999), and norepinephrine (Mårdh et al. 1985). The $\beta\beta$ allozymes may display strikingly different kinetic properties with metabolites of the biogenic amines. (c) Potential functional polymorphisms of the high-K_m class II $\pi\pi$ ADH may interact with *ADH2* to influence ethanol metabolism in the liver (Li et al. 1977) and/or the biogenic amine metabolism in the brain (Consalvi et al. 1986; Mårdh et al. 1986; Svensson et al. 1999), although functional polymorphism of $\pi\pi$, as well as its localization in the brain, have not yet been described. (d) Other candidate genes for alcoholism and *ADH2* may have functional interaction because of the presence of allelic variations, like the tryptophan hydroxylase gene (Nielsen et al. 1998). Recently, genome-wide surveys of the families of alcoholic probands have provided evidence suggestive of a protective locus on chromosome 4, affecting the risk for alcohol dependence, that includes the *ADH* gene cluster in both white and American Indian populations (Long et al. 1998; Reich et al. 1998). Association between *ADH2*3* and alcoholism in black populations has not been reported, although association between *ADH2*2* and alcoholism in other racial groups has. Further studies of various ethnic groups are needed to elucidate the underlying mechanisms by which the allelic variations at *ADH2* affect predisposition to alcoholism.

In conclusion, the functional polymorphisms at the *ADH2*, *ADH3*, and *ALDH2* exhibit a complex pattern of influences on susceptibility to alcoholism. The observed differences in frequency of *ADH3* in alcoholics and controls can be accounted for by its disequilibrium with the *ADH2*. In the *ALDH2*1/*1* homozygotes, protection by the two copies of *ADH2*2* is ~2 times stronger than that afforded by a single copy of the allele. In examining the combined effects of the *ADH2* and *ALDH2* genotypes, protection by *ADH2*1/*2-ALDH2*1/*1* appears not to be significantly different from that by *ADH2*1/*1-ALDH2*1/*2*. *ADH2*1/*2-ALDH2*1/*2* and *ADH2*2/*2-ALDH2*1/*2* exhibit 20- to 25-fold less risk for alcoholism than *ADH2*1/*1-ALDH2*1/*1*. *ALDH2*2/*2* homozygosity fully protects against the disease, regardless of the presence of the *ADH2* polymorphism. The variant *ADH2*2* allele appears to protect against alcoholism by a mechanism or mechanisms independent of that by which *ALDH2*2* protects against it, a finding that requires further explanation.

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