Effect of the allelic variants of aldehyde dehydrogenase *ALDH2**2* and alcohol dehydrogenase *ADH1B**2* on blood acetaldehyde concentrations

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Abstract

Alcoholism is a complex behavioural disorder. Molecular genetics studies have identified numerous candidate genes associated with alcoholism. It is crucial to verify the disease susceptibility genes by correlating the pinpointed allelic variations to the causal phenotypes. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the principal enzymes responsible for ethanol metabolism in humans. Both *ADH* and *ALDH* exhibit functional polymorphisms among racial populations; these polymorphisms have been shown to be the important genetic determinants in ethanol metabolism and alcoholism. Here, we briefly review recent advances in genomic studies of human *ADH*/*ALDH* families and alcoholism, with an emphasis on the pharmacogenetic consequences of venous blood acetaldehyde in the different *ALDH2* genotypes following the intake of various doses of ethanol. This paper illustrates a paradigmatic example of phenotypic verifications in a protective disease gene for substance abuse.

Keywords: *alcohol dehydrogenase*, *aldehyde dehydrogenase*, *single nucleotide polymorphism*, *alcoholism*, *ethanol metabolism*, *blood acetaldehyde*

Introduction

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the principal enzymes responsible for hepatic metabolism of ethanol.[1] Human *ADH* and *ALDH* constitute complex gene families, exhibiting functional polymorphisms among racial populations. The variant gene alleles *ADH1B*2* and *ALDH2*2* have been consistently documented to reduce the risk of developing alcohol dependence across ethnic groups.[2–4] Alcoholism is a multifactorial, polygenic behavioural disorder involving complex gene–gene and gene–environment interactions.[5,6] The pharmacological and toxicological effects of ethanol are dependent on the duration of exposure and the concentrations of ethanol and its metabolite acetaldehyde attained in body fluids and tissue within that period. Recent pharmacogenetic and pharmacodynamic studies support the notion that full protection against alcoholism in *ALDH2*2/*2* homozygotes may derive from either abstinence or deliberate moderation in alcohol consumption. This might be due to prior experience with strong unpleasant reactions caused by persistently elevated blood acetaldehyde after ingesting a small amount of alcohol. In addition, these studies have shown that partial protection against the disease in *ALDH2*1/*2* heterozygotes can be ascribed to the significantly

lower acetaldehyde accumulation in blood than is observed in variant homozygotes after low to moderate intake of alcohol.[7] This paper focuses on recent advances in genomic studies of human ethanol metabolism and alcoholism, highlighting the phenotypic manifestations with blood acetaldehyde in correlation with the pertinent allelic variations of alcohol metabolic genes.

Genetics of ADH/ALDH and alcoholism

The genes of the human *ADH* family cluster in a region of chromosome 4q21 spanning ~370 kilobases (kb). The family members have been classified into five groups, designated *ADH1–ADH5*, on the basis of their structural and functional characteristics.[2,8] Positional identities of the amino acid sequence range from 60 to 70 per cent between classes and over 90 per cent within the class. Class I–IV ADH isozymes display markedly different kinetic properties for ethanol oxidation (Table 1). Catalytic functions of class V ADH remain unknown owing to its extremely labile activity. ADH1B allozymes differ from each other by a single amino acid, resulting from a single nucleotide polymorphism (SNP) — ADH1B1 (Arg47, Arg369), ADH1B2 (His47, Arg369) and ADH1B3 (Arg47, Cys369). ADH1C allozymes exhibit two amino acid exchanges (Arg271 and Ile349 for ADH1C1; Gln271 and Val349 for ADH1C2) owing to the separate corresponding SNPs. X-ray crystallographic studies indicate that the amino acid residues 47, 271 and 369 are located at the coenzyme binding site and can influence the catalytic function of the allozymes.[11] Distribution of the functional polymorphisms of *ADH1B* and *ADH1C* genes show ethnic distinctions.[2,12,13] The *ADH1B*1* allele is prevalent among Caucasians and Native Americans. *ADH1B*2* is predominant among East Asians, including Han Chinese, Japanese and Koreans, and is also relatively high among Filipinos, Malays and the aborigines of Taiwan, Australia and New Zealand. *ADH1B*3* is found in black populations and Native Americans.

The human *ALDH* superfamily comprises ten families, mapped to 11 chromosomes.[14,15] Amino acid sequence identities are approximately 40 per cent between families and approximately 60 per cent or higher between subfamilies. The superfamily contains divergently related enzymes that metabolise a wide spectrum of endogenous and exogenous aldehydes. Based on kinetic mechanisms for catalysis, ALDH1A1, ALDH2 and ALDH3A1 are the first representatives of the class I, II, and III ALDHs, respectively. ALDH1A1 and ALDH2 primarily contribute to the *in vivo* metabolism of acetaldehyde, a direct metabolite of ethanol (Table 1). ALDH1B1 may also participate in acetaldehyde oxidation but the kinetic parameters have yet to be determined.[16] There are two ALDH2 allozymes — ALDH2E (also designated E_4, where E stands for normal type subunit Glu487) and ALDH2K (K_4, where K stands for variant type subunit Lys487). The ALDH2 allozymes exhibit an 800-fold difference in catalytic efficiency, V_{max}/K_m, for acetaldehyde oxidation. A molecular model for partial dominance of the variant ALDH2 subunit in the tetrameric molecule has been proposed.[17,18] The model is based on the dimer-of-the-dimers structure and the half-of-the-site reactivity of genetically engineered recombinant enzymes. The KK dimer in the tetrameric molecule appears to be inactive in the cell owing to the extremely high K_m for nicotinamide adenine dinucleotide (NAD) and the diminished V_{max}. The EK dimer appears to be inactive owing to the dominance of the K subunit over the E subunit through half-of-the-site reactivity. Assuming that the association of the E and K subunits is random, a mixture of the homo- and heterotetrameric molecules in *ALDH2*1/*2* heterozygotes can be described by the binomial expansion $E_4 : E_3K : E_2K_2 : EK_3 : K_4 = 1 : 4 : 6 : 4 : 1$. This partial dominance model predicts that the residual activity of E_4, E_3K and E_2K_2 in heterozygotes would account for 25 per cent of the total activity in normal *ALDH2*1/*1* homozygotes. For comparison, the co-dominant and complete dominant molecular models predict that the residual activity of ALDH2 in heterozygotes would be 50 per cent and 6.25 per cent, respectively. Further

Table 1. Human ADH and ALDH family members involved in the metabolism of ethanol

Enzyme	Class	Gene locus	Allelic variant	Subunit composition	K_m (mM)	V_{max} (U/mg)	V_{max}/K_m (U/mg/mM)
ADH	I	*ADH1A*		$\alpha\alpha$	5.2	0.24	0.047
		ADH1B	*ADH1B*1*	$\beta_1\beta_1$	0.016	0.18	12
			*ADH1B*2*	$\beta_2\beta_2$	1.9	4.8	2.6
			*ADH1B*3*	$\beta_3\beta_3$	53	3.2	0.060
		ADH1C	*ADH1C*1*	$\gamma_1\gamma_1$	0.11	0.81	
			*ADH1C*2*	$\gamma_2\gamma_2$	0.061	0.47	
	II	*ADH2*		$\pi\pi$	23	0.30	0.013
	III	*ADH3*		$\chi\chi$	3,400	0.83	
	IV	*ADH4*		$\mu\mu(\sigma\sigma)$	58	11	0.19
ALDH	I	*ALDH1A1*		Tetramer	0.033	0.63	19
	II	*ALDH2*	*ALDH2*1*	Tetramer(E_4)	0.00020	0.60	3,000
			*ALDH2*2*	Tetramer(K_4)	0.0046	0.017	3.6

Enzyme activity was determined in 0.1M sodium phosphate, at pH 7.5 and 25°C. Samples contained 0.5 mM nicotinamide adenine dinucleotide (NAD; ie a cytosolic oxidised coenzyme concentration in hepatocytes), and various concentrations of ethanol for alcohol dehydrogenase (ADH) or of acetaldehyde for aldehyde dehydrogenase (ALDH). $\gamma_1\gamma_1/\gamma_2\gamma_2$ and $\chi\chi$ exhibit negative and positive cooperativity, respectively; hence they do not have a K_m and the values shown are $S_{0.5}$. Enzyme activity units (U) are expressed as micromoles NADH formed per minute under the assay conditions. Data are from Yin and Agarwal,[1] Yin *et al.*[9] and Lee *et al.*[10]

studies are needed to compare the activities and protein content of ALDH2 in human liver tissues with three different *ALDH2* genotypes, to corroborate the proposed molecular mechanism for the negative dominance of *ALDH2*2*. The glutamic acid/lysine exchange at position 487 is caused by a single nucleotide G/A transition in exon 12 of *ALDH2* on chromosome 12. The allele frequencies of variant *ALDH2*2* are 16–35 per cent in the Han Chinese, Japanese, Koreans and Vietnamese, and 1–10 per cent in Tibetans, Mongolians, Thais, Malays, Filipinos and Taiwanese aborigines.[3,12] *ALDH2*2* rarely occurs in other ethnic groups, for example, Caucasians, black populations and American Indians.

To date, the most widely replicated candidate genes that contribute to alcoholism susceptibility are polymorphisms in the alcohol-metabolising enzymes — that is, *ADH1B* and *ALDH2*.[1–3] Homozygosity of variant allele *ADH1B*2 per se* can reduce the risk of alcohol dependence by eight-fold in East Asians,[19] whereas *ALDH2*2* homozygosity appears to be almost completely protective against the disease.[20–22] Multiple logistic regression analyses suggest that *ADH1B*2* and *ALDH2*2* may independently influence vulnerability to alcoholism.[19,21,22] The variant *ADH1B*3* allele has recently been shown to have a significant protective effect on the risk for alcoholism in African-Americans and Native Americans.[23,24] The *ADH1C*1* allele, which is in linkage disequilibrium with *ADH1B*2*, does not appear to have independent protective effects against alcoholism in East Asians.[19,25] The null effect is compatible with a much smaller difference in V_{max} for ADH1C allozymes, compared with the difference between *ADH1B* allozymes (Table 1). Recent genetic association and linkage studies have screened a large number of SNPs, most in the non-coding sequences (intronic, gene-flanking and intergenic) and only a few in the coding sequences across the entire region containing the seven *ADH* genes, suggesting that in addition to *ADH1B*, *ADH1A*, *ADH2*, *ADH3* and *ADH4* also affect the risk for alcoholism.[23,26,27] The contribution of individual candidate *ADH* genes may be small and an interaction between the genes may also exist, influencing disease

susceptibility. A functional window has been proposed for assessing protection against alcoholism by allelic variations of the *ADH* family genes, which is based on hepatic activity differences for ADH1B allozymes and for ADH1C allozymes as the thresholds of effective protection and null protection, respectively.[28] Numerous SNPs have been identified in the genes of human *ALDH1A1* and *ALDH2*.[15] Thus far, *ALDH2*2* is the only variant that has been proven to be causally associated with alcoholism.[3,29–31]

Pharmacogenetics of ethanol metabolism

The liver is the major organ for ethanol metabolism.[1,16,32] Acetaldehyde concentrations in hepatocytes depend on both the rate of generation (ie ethanol oxidation by ADHs) and the rate of removal (ie acetaldehyde oxidation by ALDHs). The functional polymorphic variants of *ADH/ALDH* genes can alter the metabolic balance of acetaldehyde. It is generally accepted that the high-activity ADH isozymes/allozymes accelerate acetaldehyde production, while the low-activity ALDH2 allozymes slow down its degradation. Cytosolic class I ADH1A, 1B, 1C, class II and III ADHs, as well as cytosolic ALDH1A1 and mitochondrial ALDH2, participate in human hepatic ethanol metabolism. The relative contributions of ADH/ALDH isozymes and allozymes are primarily determined by the respective kinetic parameters, the tissue isozyme composition and contents, and the cellular ethanol/acetaldehyde concentrations.[10,33] At low ethanol levels, ADH1B (except for 1B3) and ADH1C isozymes/allozymes are active in the liver owing to the low K_ms (Table 1). At higher ethanol levels, the high-K_m ADH1A, ADH1B3 and ADH2 become active. ALDH2 plays a major role in the hepatic oxidation of acetaldehyde. This is mainly based on its lowest K_m for acetaldehyde (0.20 μM) (Table 1) and the subcellular location that is directly linked to the electron transport system coupled with oxidative phosphorylation in the mitochondria. Cytosolic ALDH1A1 may also contribute to acetaldehyde catabolism, since its K_m (33 μM) is comparable with peak acetaldehyde levels (~15 μM) in hepatic venous blood after ingestion of 0.8 g/kg ethanol.[34] Gastric first-pass metabolism can influence the bioavailability of ethanol.[32] ADH1C, ADH3 and ADH4 are expressed in the human stomach and may contribute to ethanol oxidation with quantitative significance.[10,33,35]

The functional polymorphism of *ALDH2* dramatically influences the blood acetaldehyde concentration in the cubital vein in East Asians after alcohol consumption (Table 2). For the homozygous *ALDH2*2/*2* genotype, the peak blood acetaldehyde concentrations are 1.6–3.3-fold higher than in *ALDH2*1/*2* heterozygotes following a low to a moderate intake of ethanol. Both homozygotes and heterozygotes show increased blood acetaldehyde when the ethanol dose increases, indicating a more rapid formation of acetaldehyde with higher alcohol intake. The peak blood alcohol concentrations after ingestion of 0.2 g/kg and 0.5 g/kg ethanol in *ALDH2*2* homozygotes are 4.1 mM and 12 mM, respectively.[21,29] The increased ethanol oxidation rate at higher alcohol concentrations can be attributed to the contributions of hepatic high-K_m ADH1A and ADH2.[10,33] It is worth noting that cytosolic ALDH1A1 is mainly responsible for acetaldehyde oxidation in the variant *ALDH2*2* homozygotes because of a lack of ALDH2 activity. In heterozygotes, the hepatic residual ALDH2 activity also contributes significantly to the degradation of acetaldehyde. This could explain the much lower blood acetaldehyde found in heterozygotes than in variant homozygotes. Notably, at 130 minutes after drinking a relatively small amount of ethanol (0.2 g/kg), variant homozygotes still exhibit blood acetaldehyde levels (17 ± 3 μM; mean ± SE) similar to that of the peak concentrations (24 ± 1 μM) of the heterozygotes.[29] This suggests that the full protection against alcoholism by *ALDH2*2/*2* homozygosity can be pharmacogenetically ascribed to a prolonged and large accumulation of acetaldehyde in the blood, which results from an almost complete loss of ALDH2 activity in the liver. After challenge with a moderate dose of ethanol (0.5 g/kg), however, heterozygotes can exhibit a comparable blood acetaldehyde level (76 μM) to that of variant

Table 2. Venous blood acetaldehyde concentrations in male adults with different *ADH* and *ALDH* genotypes after alcohol ingestion

Ethanol dose (g/kg)	Combinatorial genotype		Subject number	Mean peak acetaldehyde	
	ALDH2	*ADH1B*	(*n*)	(μM)	Author
0.2	*1/*1	*2/*2	6	1.0	Peng *et al.*[29]
0.2	*1/*2	*2/*2	6	24	Peng *et al.*[29]
0.2	*2/*2	*2/*2	6	75	Peng *et al.*[29]
0.3	*1/*1	*1/*1	6	0.3	Peng *et al.*[30]
0.3	*1/*1	*2/*2	6	0.2	Peng *et al.*[30]
0.3	*1/*2	*1/*1	6	60	Peng *et al.*[30]
0.3	*1/*2	*2/*2	6	60	Peng *et al.*[30]
0.4	*1/*1	*1/*1	5	3.3	Mizoi *et al.*[36]
0.4	*1/*1	*1/*2	8	4.8	Mizoi *et al.*[36]
0.4	*1/*1	*2/*2	20	4.2	Mizoi *et al.*[36]
0.4	*1/*2	*1/*1	4	23	Mizoi *et al.*[36]
0.4	*1/*2	*1/*2	9	23	Mizoi *et al.*[36]
0.4	*1/*2	*2/*2	16	24	Mizoi *et al.*[36]
0.4	*2/*2	*2/*2	6	79	Mizoi *et al.*[36]
0.5	*1/*1	*1/*1	8	3.3	Peng *et al.*[31]
0.5	*1/*1	*2/*2	8	4.0	Peng *et al.*[31]
0.5	*1/*2	*2/*2	16	76	Peng *et al.*[31]
0.5	*2/*2	*2/*2	1[a]	125	Chen *et al.*[21]

[a]An alcohol-dependent patient with normal liver function.
Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase

homozygotes (75 μM) receiving 0.2 g/kg ethanol.[29,31] This may explain the partial protection against alcoholism by *ALDH2*1/*2* heterozygosity, being due to a significantly faster elimination and hence lower accumulation of acetaldehyde resulting from the residual activity of hepatic ALDH2, thus allowing heterozygotes to tolerate small amounts of alcohol. Interestingly, following alcohol ingestion in East Asians and Caucasians with the normal *ALDH2*1/*1* genotype, blood acetaldehyde concentrations are near or below the detection limit.[29–31,36,37] This reflects that ALDH2 and ALDH1A1 can efficiently oxidise most of the acetaldehyde derived from ethanol in the liver, and that peripheral tissues, including red blood cells, can eliminate trace amounts of acetaldehyde entering the circulation in normal homozygotes. Although the ADH1B2 allozyme exhibited a higher activity for ethanol oxidation, and individuals carrying the *ADH1B*2* allele showed higher alcohol elimination rates,[10,38] combinatorial genotype studies demonstrate that *ADH1B* polymorphism does not reveal significant differences in blood acetaldehyde levels in *ALDH2*1/*1* or *ALDH2*1/*2* genotypes (Table 2). This suggests that the increased generation of acetaldehyde from hepatic ADH1B2 can be effectively metabolised by the hepatic and extrahepatic low-K_m ALDHs. Notably, the *ADH1B* polymorphism *per se* does not

lead to an appreciable blood acetaldehyde accumulation in normal *ALDH2*1/*1* homozygotes. The physiological basis for protection by the *ADH1B* variant against alcoholism remains largely unknown and it warrants further investigation.

Summary and future perspectives

The allelic variation of *ALDH2* has been shown to be a unique functional polymorphism, which results in loss of enzyme activity in a partially negative dominant fashion. The severity of the inborn error of acetaldehyde metabolism differs markedly between homozygosity and heterozygosity of the variant *ALDH2*2* allele. The *ALDH2* polymorphism, which commonly occurs in East Asian populations, is, as yet, the strongest genetic modifier of drinking behaviour and risk for alcoholism. The genotypes of *ALDH2*2* can be causally correlated with the pharmacogenetic and pharmacodynamic phenotypes following intake of various doses of ethanol, providing the first example of functional verification of disease genes for substance abuse. There remain several critical issues that need to be addressed in future human or animal studies: (i) quantitative correlation of acetaldehyde concentrations in the peripheral blood, hepatic venous blood and cerebrospinal fluid in different *ALDH2* genotypes following ethanol intake; (ii) quantitative assessment of the contributions of cytosolic ALDH1A1 and mitochondrial ALDH2 in hepatic and extrahepatic tissues under various ethanol/acetaldehyde concentrations; (iii) modelling with the kinetic mechanism-based rate equations and prediction of hepatic ethanol metabolism, particularly the steady-state concentrations of acetaldehyde, by the complex myriad of ADH/ALDH families.

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