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Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology

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Alcohol dehydrogenase (ADH) and mitochondrial aldehyde dehydrogenase (ALDH2) are responsible for metabolizing the bulk of ethanol consumed as part of the diet and their activities contribute to the rate of ethanol elimination from the blood. They are expressed at highest levels in liver, but at lower levels in many tissues. This pathway probably evolved as a detoxification mechanism for environmental alcohols. However, with the consumption of large amounts of ethanol, the oxidation of ethanol can become a major energy source and, particularly in the liver, interferes with the metabolism of other nutrients. Polymorphic variants of the genes for these enzymes encode enzymes with altered kinetic properties. The pathophysiological effects of these variants may be mediated by accumulation of acetaldehyde; high-activity ADH variants are predicted to increase the rate of acetaldehyde generation, while the low-activity ALDH2 variant is associated with an inability to metabolize this compound. The effects of acetaldehyde may be expressed either in the cells generating it, or by delivery of acetaldehyde to various tissues by the bloodstream or even saliva. Inheritance of the high-activity ADH β2, encoded by the *ADH2*2* gene, and the inactive *ALDH2*2* gene product have been conclusively associated with reduced risk of alcoholism. This association is influenced by gene–environment interactions, such as religion and national origin. The variants have also been studied for association with alcoholic liver disease, cancer, fetal alcohol syndrome, CVD, gout, asthma and clearance of xenobiotics. The strongest correlations found to date have been those between the *ALDH2*2* allele and cancers of the oro-pharynx and oesophagus. It will be important to replicate other interesting associations between these variants and other cancers and heart disease, and to determine the biochemical mechanisms underlying the associations.

Alcohol dehydrogenase: Aldehyde dehydrogenase: Liver: Cancer

Alcohol is used by a large number of individuals and its metabolism parallels that of other nutrients. While the use of small amounts of alcohol has a beneficial effect for cardiovascular health, consumption of large amounts has well-known effects on the liver, heart, pancreas and the nervous system, and less well-recognized influences on other disease, especially cancers. The susceptibility of individuals to the ill effects of alcohol consumption appears to be a result of complex interactions between genes and the environment (the latter including both the alcohol itself and other nutrients). The enzymes involved in alcohol metabolism are polymorphic and it is their contribution to differential risk of alcoholism and some of its complications that is most understood. These enzymes and the effects of their genetic variation are the subject of the present review.

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AUC, areas under the blood alcohol concentration curves; C/EPB, CCAAT-enhancer-binding proteins; FPM, first-pass metabolism; V_{max}, maximum velocity.

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Gastrointestinal absorption and first-pass metabolism

First-pass metabolism (FPM) is the difference between the amount of a drug administered orally and the amount reaching the systemic circulation and, conceptually, is a result of metabolism of the drug by the gut or liver during the absorption phase. FPM is important because it reduces the amount of a drug reaching target organs and may also predispose gut tissues to injury from alcohol metabolism. The gastrointestinal tract, similar to the liver, contains cytochrome P450s and alcohol dehydrogenases (ADH). Ingested ethanol is absorbed slowly from the stomach, during which process it may be subject to oxidation. Ethanol leaving the stomach is very rapidly absorbed from the upper small intestine. Ethanol absorbed across the gut mucosa is carried to the liver by the portal vein, where a small proportion is metabolized before leaving that organ. To determine the magnitude of FPM, ethanol is administered orally or intravenously and the concentration of ethanol in the blood (blood alcohol concentration) is measured over time. The areas under the blood alcohol concentration curves (AUC) are calculated for each route of administration and the FPM is the difference in AUC between the two routes (Julkunen *et al*. 1985; Caballeria *et al*. 1987).

Lieber's group pioneered studies on FPM, establishing that FPM is easiest to detect with low doses of ethanol (0·3 g/kg, equivalent to approximately 20 g ethanol or two social drinks) when gastric emptying is slowed by the presence of food (Julkunen *et al*. 1985). Larger doses of ethanol or conditions under which gastric emptying is rapid make the difference between the AUC too small to measure accurately. While the phenomenon of FPM is well established, the organ in which it occurs is not. Some reports favour the stomach as a major site (Lim *et al*. 1993). The gastric mucosa contains several ADH (γ-ADH, χ-ADH and σ-ADH, see p. 51) that could be involved in the metabolism of ethanol. Under circumstances in which gastric ADH activity is decreased, e.g. in women (Frezza *et al*. 1990; Seitz *et al*. 1993), individuals with atrophic gastritis, alcoholics (DiPadova *et al*. 1987) and individuals taking certain medications (Roine *et al*. 1990; Cabelleria *et al*. 1991), the magnitude of FPM is reduced. σ-ADH, a major gastric ADH isozyme, is absent in the stomach biopsies of about 30% of Asians, and those lacking this enzyme had lower FPM of ethanol (Dohmen *et al*. 1996), suggesting that σ-ADH is important in gastric oxidation of ethanol. The relationship between FPM and the rate of gastric emptying suggests that prolonged contact of the ingested alcohol solution with the stomach favours absorption of the alcohol transgastrically, where it would be subject to oxidation in the stomach mucosal cells. Rapid gastric emptying would have the opposite effect. Oral administration of alcohol resulted in a substantially higher blood alcohol level and AUC in the fasted as compared with the fed state (DiPadova *et al*. 1987). All these findings are consistent with an important role for the stomach mucosa in FPM of ethanol.

However, other interpretations of these data have been published. The assertion that gastric ADH (Yin *et al*. 1997) or FPM (Ammon *et al*. 1996) is reduced in women is contested. Some investigators have found no correlation between gastric ADH activity and FPM (Brown *et al*. 1995). Importantly, the total ADH activity in the stomach, calculated based on the mass of the mucosa and its ADH activity, does not account for the amount of ethanol metabolized, as indicated by the observed differences between the AUC of oral and intravenous alcohol (Yin *et al*. 1997). Furthermore, the human and rat σ-ADH have markedly different kinetic properties. The K_m for ethanol for the human enzyme is 40 mM, whereas that for the rat enzyme is eighty times greater, yet FPM for the two species is similar in magnitude. These arguments suggest that FPM also occurs in the liver. Hepatic FPM is dependent on the rate at which ethanol is absorbed, since at low rates of absorption, leading to low portal venous ethanol concentrations, ethanol could be extracted by the relatively-low-K_m hepatic ADH isozymes. At higher rates of absorption and higher portal ethanol concentrations, these enzymes will be saturated. Experimentally, the systemic AUC of ethanol concentration is very sensitive to the rate of portal venous administration of ethanol (Smith *et al*. 1992; Levitt & Levitt, 1994). This alternative explanation can also account for the lack of FPM seen with high doses of ethanol or rapid gastric emptying.

To clarify this issue, Ammon *et al*. (1996) gave ethanol intravenously and ^{2}H-labelled ethanol by mouth or into the duodenum. This method reduced the intra-subject variability by permitting an estimate of both gastric and hepatic FPM simultaneously. They found that FPM was about 8–9% of the oral dose and estimated that the gastric contribution to FPM was about 6% of the oral dose. It seems safe to conclude that the FPM of oral ethanol is usually a small percentage (perhaps ≤10) of the total body ethanol elimination, and when gastric emptying is rapid or the ethanol dose consumed is high it is quantitatively even less important. Gender differences in FPM are probably not major (Ammon *et al*. 1996). The overall importance of FPM might lie in the potential for gastric FPM to protect the liver and other organs from low doses of ethanol, and for certain drugs to block FPM (Roine *et al*. 1990; Caballeria *et al*. 1991), resulting in intoxication from smaller-than-expected doses of ethanol. Furthermore, this process is an obvious point of intersection of diet and timing of meals with ethanol consumption.

Overview of hepatic ethanol metabolism and its regulation

After absorption and passage through the liver, ethanol is distributed in the body water space and is largely metabolized in the liver to acetaldehyde by ADH in the cytosol and the cytochrome P450IIE1 in microsomes. Although cytochrome P450IIE1 is important in ethanol toxicity and in mediating several drug–ethanol interactions, it will not be further considered in the present discussion. Acetaldehyde is converted by aldehyde dehydrogenases (ALDH) (especially the mitochondrial ALDH2 isozyme) to acetate, which is released from the liver and metabolized by the heart and muscle (Lumeng & Davis, 1970). The rate of ethanol metabolism by ADH and ALDH2 may be critical in determining its toxicity because the intermediates of this

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pathway are themselves potentially toxic. The maximal activities of ADH and ALDH in the liver are similar, so that each enzyme contributes to the overall control of the rate of alcohol oxidation.

Modelling of alcohol oxidation in rat liver indicated that ADH activity was controlled in part by the total activity of the enzyme as well as product inhibition by NADH and acetaldehyde (Crabb *et al*. 1983). Liver NADH levels are elevated during alcohol oxidation because the first enzyme in the malate–aspartate shuttle, malate dehydrogenase, has a high K_m for NADH (Crow *et al*. 1982, 1983). Thus, in a steady-state ADH is operating below its maximum velocity (V_{max}). Flux through the pathway is also sensitive to the total activity of ADH. Reduction in total ADH activity (as occurs in fasting) reduced the ability of the liver to oxidize ethanol in rats, but increases in activity did not increase the metabolic rate proportionally (Crabb *et al*. 1983). This outcome is presumed to be a result of the inability to increase acetaldehyde oxidation and, therefore, an increase in steady-state acetaldehyde concentration may limit the rate of ethanol metabolism. In human subjects with ADH and ALDH2 variants with markedly different kinetic properties the rate of ethanol oxidation should be influenced by the K_m, V_{max} and sensitivity to product inhibition of the variants. This relationship raises the possibility that under certain conditions pathways of alcohol metabolism and the concentrations of metabolic intermediates change but the alcohol elimination rate does not.

The rate of ethanol clearance from the blood in the pseudo-linear segment of the elimination curve varies by two- to threefold between individuals (Kopun & Propping, 1977; Martin *et al*. 1985). The test–retest reliability in the oral ethanol challenge method of determining the alcohol elimination rate is open to criticism, but substantial between-individual variation was recently confirmed using the alcohol clamp technique (O'Connor *et al*. 1998), which completely avoids the variability in absorption. The reasons for this variation are incompletely understood. The likeliest explanation for the difference is variation in the activity of enzymes catalysing alcohol oxidation and in the size of the liver. Examination of these isozymes and their genes has revealed a substantial number of functional polymorphisms, which contribute to responses to ethanol.

Enzymology of alcohol metabolism

Alcohol dehydrogenases

The enzymes responsible for the bulk of alcohol oxidation are the ADH. All are dimeric Zn-containing enzymes with a subunit molecular weight of 40 kDa. These enzymes are classified based on enzymic properties and the extent of sequence similarities. Only enzyme subunits belonging to the same class can heterodimerize. The heterodimers have kinetic properties described by the active sites acting independently. Classes I, II and possibly IV are predicted to participate in ethanol oxidation *in vivo*. The properties of the enzymes in each of these classes are summarized in Table 1 (Bosron & Li, 1986, 1987). Class I contains α, β and γ isozymes. These enzymes have a low K_m for ethanol and are highly sensitive to inhibition by pyrazole derivatives. Class I enzymes are very abundant in the liver and are therefore believed to play a major role in hepatic alcohol metabolism. Class II ADH (π ADH, for pyrazole-insensitive isozyme) was first found in human liver; it has a higher K_m for ethanol and is less sensitive to pyrazole inhibition than class I enzymes (Ehrig *et al*. 1990). As it has a high K_m it may contribute to increased rates of alcohol elimination (i.e. a steeper blood ethanol disappearance curve) sometimes observed at high blood ethanol concentrations. Class III ADH (χ ADH) is expressed in all tissues studied, is virtually inactive with ethanol but is capable of metabolizing longer-chain alcohols and ω-hydroxy-fatty acids (Pares & Vallee, 1981). This enzyme also exhibits glutathione-dependent formaldehyde dehydrogenase activity (Koivusalo *et al*. 1989).

Recent additions to this family of enzymes are class IV and (tentatively) classes V and VI. The class IV enzyme has been purified from the stomach and oesophagus.

Table 1. Properties of alcohol dehydrogenases (ADH) in man

Gene locus	New nomenclature	Subunit type	K_m (ethanol)†	V_{max}†	Tissue distribution
Class I					
ADH1	*ADH1A*	α	4	54	Liver
ADH2	*ADH1B*	β	0·05–34·0‡	–	Liver, lung
ADH3	*ADH1C*	γ	0·6–1·0‡	–	Liver, stomach
Class II					
ADH4	*ADH2*	π	34	40	Liver, cornea
Class III					
ADH5	*ADH3*	χ	1000	–	Most tissues
Class IV*					
ADH7	*ADH4*	σ, μ	20	1510	Stomach, oesophagus, other mucosas
Class V*					
ADH6	*ADH5*	–	30	?	Liver, stomach
Class VI*					
ADH8	*ADH6*	–	–		Not detected in man, found in deer mouse and rat liver

V_{max}, maximum velocity.
*Tentative assignments based on sequence homologies. Details for the class IV, V and VI enzymes are given on pp. 51–52.
†K_m values are given in mM and V_{max} values are given in terms of turnover number (/min).
‡Kinetic constants vary with the isozyme, see Table 2.

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Designated either μ-ADH (for the mucosal isozyme; Yin *et al*. 1990) or σ-ADH (for the stomach isozyme; Moreno & Pares, 1991), it is structurally distinct from classes I, II and III (Stone *et al*. 1993; Farres *et al*. 1994*a*; Pares *et al*. 1994; Satre *et al*. 1994). Its high K_m for ethanol may be an adaptation for the high concentration of ethanol in the gastric mucosa after ethanol consumption. The fact that this enzyme has such a high K_m for ethanol suggests that other alcohols may be its physiological substrates. σ-ADH has the highest V_{max} of any of the known ADH and is very active with retinol as substrate (Stone *et al*. 1993). Its expression in a variety of epithelia (oesophagus, stomach, vagina, naso-pharynx and cornea) and the importance of retinol in the integrity of these tissues suggest that σ-ADH has a role in retinol conversion to retinal. It is the first ADH expressed in the embryonic mouse and its sites of expression correlate with the production of retinoic acid (Ang *et al*. 1996*a*,*b*). Class V ADH, encoded by the *ADH6* gene, is expressed at the mRNA level in the liver and in the stomach, but the enzyme itself has not been purified. *In vitro* expressed enzyme had an isoelectric point of about 8·6, a high K_m for ethanol (about 30 mM) and moderate sensitivity to pyrazole inhibition (Cheng & Yoshida, 1991; Yasunami *et al*. 1991). An additional class of ADH (tentatively designated class VI) was reported in the liver of deer mice (*Peromyscus maniculatus*; Zheng *et al*. 1993) and rats (Hoog & Brandt, 1995) and class VII ADH was cloned from chicken (Kedishvili *et al*. 1997); to date the human homologues have not been found. There is a new nomenclature for ADH, shown in Table 1, but the remainder of the present review will use the older system.

The class I enzymes and their mRNA are quite abundant in liver. The genes are approximately 15 kb in size with nine exons (Duester *et al*. 1986). The ADH promoters contain binding sites for general transcription factors (e.g. TATAA-binding factors, upstream stimulatory factor (Potter *et al*. 1991*a*), CCAAT box-binding transcription factor, or nuclear factor 1, which appears to function as a negative factor (Edenberg *et al*. 1993), and Sp1-like factors (Brown *et al*. 1992)) as well as tissue-specific factors (e.g. hepatocyte nuclear factor 1, D-box-binding protein and CCAAT-enhancer-binding proteins (C/EBP; C/EBP α and β; Stewart *et al*. 1990, 1991; Potter *et al*. 1991*b*)). Exceptions are the *ADH5* and *ADH7* promoters, which lack TATAA boxes. The *ADH5* promoter is G + C rich, a characteristic of housekeeping genes.

ADH are expressed in a variety of extrahepatic tissues, albeit at lower levels than in the liver. High levels of class I ADH mRNA were found in the kidney, stomach, duodenum, colon and uterus of rats (Estonius *et al*. 1993), with lower levels in many organs, including the lung, small intestine and hepatic Ito cells (Yamauchi *et al*. 1988). Very low levels were found in the brain, thymus, muscle or heart (Estonius *et al*. 1993). Class I ADH has also been found in blood vessels (Allali-Hassani *et al*. 1997), a finding relevant to the alcohol-induced flush reaction. Surveys of ADH expression patterns in human tissue have been published (Engeland & Maret, 1993; Estonius *et al*. 1996). The expression of ADH in gut mucosa and breast is relevant to studies on the effect of alcohol on cancers of these organs. The oral mucosa expresses σ and χ ADH (Dong *et al*. 1996). Class II ADH was detected in liver and duodenum (Estonius *et al*. 1993). σ ADH is expressed in stomach and oesophageal mucosa at high levels. The colon expresses γ ADH in the mucosa and β ADH in the muscle layer (Yin *et al*. 1994). Breast tissue expresses relatively high levels of class I ADH (Triano *et al*. 2003), but the isozyme involved is not known. Enzyme extracted from breast was apparently saturated at 10 mM ethanol, which would be consistent with either β or γ ADH. Human placenta expresses χ ADH only (Pares & Vallee, 1981).

The expression of ADH is regulated to a certain extent in the liver. Binding sites for thyroid hormone, retinoic acid (Duester *et al*. 1991; Harding & Duester, 1992) and glucocorticoid receptors (Winter *et al*. 1990) have been identified in the upstream regions of class I *ADH* genes. *In vitro* promoter studies suggest that the genes are regulated (retinoic acid and glucocorticoids activating transcription and thyroid hormone antagonizing the effect of retinoic acid; Harding & Duester, 1992), but smaller effects are seen *in vivo*. This disparity may be the result of effects of the hormones on protein synthesis and turnover as well as on transcription (Qulali & Crabb, 1992; Dipple *et al*. 1993). Growth hormone increased ADH activity in intact animals and cultured hepatocytes (Mezey & Potter, 1979; Mezey *et al*. 1986*b*; Potter *et al*. 1989, 1993), while androgens (Mezey *et al*. 1986*a*) and thyroid hormones (Mezey & Potter, 1981; Dipple *et al*. 1993) decreased it. Liver ADH activity is also decreased substantially by fasting (Bosron *et al*. 1984) and protein restriction (Lumeng *et al*. 1979).

The effect of ethanol on ADH expression is complex. Studies in rodents have shown that ethanol can increase ADH activity in male rats by reducing testosterone levels (Rachamin *et al*. 1980). The doses of ethanol delivered to rats or mice via the use of liquid diets does not have significant effects on liver ADH. However, higher doses achieved by intragastric delivery of ethanol induced liver ADH activity and resulted in cyclic changes in blood alcohol despite continuous infusion. This effect was shown to result from induction of the transcription factor C/EBPβ and suppression of C/EBPγ and a truncated inhibitory form of C/EBPβ termed LIP (He *et al*. 2002). In addition, chronic intragastric infusion of ethanol increases portal vein endotoxin and sensitizes the liver to endotoxin actions (Enomoto *et al*. 2000). Mezey's group (Potter *et al*. 2003) reported that endotoxin can induce ADH mRNA, protein and activity. This effect was correlated with increased binding of upstream stimulatory factor to the ADH promoter. In man less is known. The amount of ADH in the liver is not influenced by chronic drinking; the activity is normal in heavy drinkers without liver disease and there is a progressive reduction in ADH activity as liver injury progresses (Panes *et al*. 1989). Although there are controversial data about women having higher alcohol elimination rates, orchiectomy increased alcohol elimination rates in human subjects (Mezey *et al*. 1988).

Of the seven human ADH gene loci, two are polymorphic, and the frequency of the different alleles depends on ethnic background. Both polymorphic alleles involve class I ADH genes; three alleles exist for *ADH3* and three for *ADH2* (Burnell & Bosron, 1989). The kinetic properties

Table 2. Properties of polymorphic forms of human alcohol dehydrogenase (ADH)*

Gene locus	Subunit type	K_m (ethanol)‡	V_{max}‡	Population§
*ADH2*1 (ADH1B*1)*	β1	0·05	9	Caucasians, African-Americans
*ADH2*2 (ADH1B*2)*	β2	0·9	400	Asians
*ADH2*3 (ADH1B*3)*	β3	34	300	African-Americans
*ADH3*1 (ADH1C*1)*	γ1	1·0	87	All groups
*ADH3*2 (ADH1C*2)*	γ2	0·63	35	Caucasians
*ADH3*3 (ADH1C*3)*†	γ3			Native Americans

V_{max}, maximum velocity.
*The kinetic constants are noted for the homodimers of the subunits listed (Bosron & Li, 1986, 1987; Ehrig *et al.* 1990). Heterodimers behave as if the active sites were independent.
†The third *ADH3* allele was recently discovered and the enzymic characteristics are unknown (Osier *et al.* 2002).
‡K_m values are expressed in mM and the V_{max} values are given in terms of turnover numbers (/min), as in Table 1.
§Populations that have high allele frequencies for these variants. The alleles are not limited to those populations.

and population distributions of these allelic enzymes are shown in Table 2. The isozymes encoded by the three *ADH2* alleles, which differ at a single amino acid residue, vary markedly in K_m for ethanol and V_{max}. β1 is most common in Caucasians, has a low V_{max} and a very low K_m for ethanol. β2, originally designated 'atypical' ADH (von Wartburg *et al.* 1964), is found in Asians and Ashkenazi Jews in Israel and the USA (Neumark *et al.* 1998). It has a substantially higher V_{max} and somewhat higher K_m compared with β1. The β3 isozyme was first detected in liver extracts from African-Americans (Bosron *et al.* 1980) because of its lower pH optimum than the other ADH isozymes. It has also been found in Southwest Native Americans. It has a high K_m for ethanol and high V_{max}. Smaller differences in enzymic properties are observed between the products of the *ADH3* alleles. The γ1 isozyme has about twice the V_{max} of the γ2 isozyme, while their K_m for ethanol are similar. γ1 ADH is found at a high frequency in Asians and African-Americans; Caucasians have about equal frequency of γ1 and γ2 ADH alleles (Burnell & Bosron, 1989). The recently described *ADH3*3* allele has not been enzymically characterized (Osier *et al.* 2002). Edenberg's laboratory (Edenberg *et al.* 1999) recently reported polymorphisms in the promoter of the *ADH7* gene, encoding π ADH, which affect promoter activity *in vitro*. The other *ADH* loci have not been found to be polymorphic to date.

It could be predicted that individuals expressing the variants of ADH2, in particular, would have different alcohol elimination rates; specifically, those with *ADH2*2* and *ADH2*3* would be predicted to metabolize ethanol more rapidly. This difference has been difficult to demonstrate, in part because a given isozyme constitutes a small proportion of the total alcohol-oxidizing capacity of the liver and because alcohol elimination rates are rather variable, even among individuals of the same *ADH* genotypes, or even twins (Kopun & Propping, 1977; Martin *et al.* 1985). To date, different *ADH2*2* genotypes have been correlated, at most, with only a small proportion of the between-individual differences in alcohol elimination rates (Mizoi *et al.* 1994). The *ADH2*3* polymorphism has been shown to be associated with a 10% increase in the rate of ethanol metabolism (Thomasson *et al.* 1995). The *ADH3* polymorphism did not affect alcohol elimination (Couzigou *et al.* 1991).

Table 3. Properties of human aldehyde dehydrogenases (ALDH)

Gene locus	Structure‡	K_m (Ach)	Tissue distribution
Class 1			
ALDH1	α4	30 μM	Many tissues, liver > kidney
Class 2			
ALDH2	α4	1 μM	Low levels in many tissues Liver > kidney > muscle > heart
*ALDH5**	?	?	Low levels in most tissues, placenta Liver > kidney > muscle
Class 3			
ALDH3	α2	11 mM	Stomach, liver, cornea
Other enzymes†			
ALDH4			Glutamate γ-semialdehyde dehydrogenase
ALDH6			Retinal dehydrogenase
ALDH7,8			Related to ALDH3
ALDH9			ALDHE3
ALDH10			Fatty aldehyde dehydrogenase

Ach, acetaldehyde.
*ALDH5 is tentatively assigned to class 2 because of sequence similarities to ALDH2 and the presence of a potential mitochondrial leader sequence.
†It is not likely that *ALDH6-10* play any role in metabolism of acetaldehyde.
‡The structure of the enzymes is indicated by α2 for dimers and α4 for tetramers.

Aldehyde dehydrogenases

Acetaldehyde is further metabolized by NAD^+-dependent ALDH (Table 3). These enzymes have broad substrate specificity for aliphatic and aromatic aldehydes, which are irreversibly oxidized to their corresponding carboxylic acids. The ALDH are expressed in a wider range of tissues than the ADH isozymes. The nomenclature for ALDH has been revised; they have been tentatively classified as class 1 (low K_m, cytosolic), class 2 (low K_m, mitochondrial) and class 3 (high-K_m ALDH, such as those expressed in tumours, stomach and cornea) based on kinetic properties and sequence similarities.

The most important enzymes for acetaldehyde oxidation are cytosolic ALDH1 and mitochondrial ALDH2 (Greenfield & Pietruszko, 1977). Both are tetrameric enzymes composed of 54 kDa subunits. ALDH1 has a low K_m for

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acetaldehyde (about 30 μM) and is exquisitely sensitive to disulfiram (Antabuse; Alpharma AS, Oslo, Norway) *in vitro* (Greenfield & Pietruszko, 1977; Dickinson *et al.* 1981). ALDH2 has a submicromolar K_m for acetaldehyde and is less sensitive to disulfiram *in vitro*. These enzymes have high inhibition constants for NADH and, thus, are not inhibited by the high NADH:NAD^+ that is established in cytosol and mitochondria during the oxidation of ethanol. The enzymes are distributed more or less evenly across the liver acinus. ALDH1 and ALDH2 mRNA are expressed in a variety of human tissues in addition to the liver (Stewart *et al.* 1996*b*); ALDH2 mRNA is particularly abundant in the kidney, muscle and heart. Low levels of ALDH1 and ALDH2 mRNA are found in the placenta, brain and pancreas, which may be relevant to the genesis of fetal alcohol syndrome, alcoholic neurotoxicity and chronic alcoholic pancreatitis. In man the oral mucosa expresses ALDH3, the oesophagus expresses ALDH1 and ALDH3, the stomach expresses ALDH1, 2 and 3, while the colon expresses predominantly ALDH1, and the lung expresses ALDH2 (Yin *et al.* 1993, 1994, 1997). The breast is reported to express ALDH1 and ALDH3 (Sreerama & Sladek, 1997) and the placenta expresses low levels of ALDH5 mRNA (Stewart *et al.* 1996*b*).

The control of expression of these enzymes has been studied. The *ALDH1* gene was cloned (Hsu *et al.* 1989) and the promoter was studied in transfection and DNA-binding assays. 5′ Flanking DNA (2·6 kb) permitted expression of reporter constructs in hepatoma cells; a minimal promoter was shown to bind nuclear factor Y/CCAAT-binding protein 1 and octamer factors (Yanagawa *et al.* 1995). The *ALDH2* gene has been more intensively studied. It has no TATAA box (Hsu *et al.* 1988); similar to ALDH1, it has a binding site for the ubiquitous CCAAT-box-binding protein nuclear factor Y/CCAAT-binding protein 1 near the transcription start site (Stewart *et al.* 1996*a*). Pinaire *et al.* (1999) described a site designated FP330-3′ that is bound and activated by hepatocyte nuclear factor-4 and retinoid X receptor, while apoA regulatory protein-1, chicken ovalbumin upstream promoter–transcription factor and PPAR-δ oppose this activation. These authors concluded that it is likely that the FP330-3′ site integrates the effects of several transcription factors in different tissues and this process may explain why ALDH2 is highly expressed in liver and kidneys.

Additional ALDH enzymes are known, but their role in alcohol metabolism is unknown. Pietruszko's laboratory (Kurys *et al.* 1989, 1993) purified and cloned an enzyme designated ALDHE3. This enzyme has properties similar to ALDH1; it is expressed in the cytosol and has a K_m for aliphatic aldehydes of about 30–50 μM (Kurys *et al.* 1989), but is only 40% similar to ALDH1 or ALDH2 at the amino acid level (Kurys *et al.* 1993). This enzyme has a low K_m for aminoaldehydes such as 4-aminobutyraldehyde and hence may metabolize compounds derived from polyamines such as spermine and betaine aldehyde (Chern & Pietruszko, 1995). Its gene (designated *ALDH9*) was recently cloned (Lin *et al.* 1996). *ALDH5* (originally known as $ALDH_x$; Hsu & Chang, 1991) is unique among the *ALDH* genes in that it lacks introns. The enzyme has 70% sequence similarity to ALDH2 and is predicted to contain a mitochondrial leader sequence. If so, ALDH5 may be classified as the second class 2 ALDH. The *ALDH5* gene is also polymorphic at two different residues: valine or alanine at position 69; leucine or arginine at position 120 (Hsu & Chang, 1991; Sherman *et al.* 1994). It is not known at present whether these substitutions alter the enzymic properties of ALDH5. The highest levels of ALDH5 mRNA are expressed in the liver, kidney and skeletal muscle (Stewart *et al.* 1996*b*).

ALDH3 and ALDH4 are abundant in liver extracts, have considerably higher K_m for aliphatic aldehydes than the class 1 and 2 enzymes and higher affinity for aromatic aldehyde substrates. The class 3 ALDH3 family includes the cytosolic, tetrachlorodibenzoparadioxin (dioxin)-inducible ALDH, the hepatoma-associated ALDH and the corneal and stomach ALDH3 (Lindahl, 1992; Algar *et al.* 1993). The stomach form might participate in the oxidation of acetaldehyde generated during gastric metabolism of ethanol. ALDH4 appears to be glutamic γ-semialdehyde dehydrogenase. ALDH6 is a retinal dehydrogenase. ALDH7 and ALDH8 have been cloned (Hsu *et al.* 1995), but enzymological characteristics for these enzymes are not yet known. They are related to ALDH3. ALDH9 represents the gene for ALDHE3, and ALDH10 encodes the fatty ALDH that is deficient in Sjogren-Larsson syndrome.

Similar to the class I ADH, the *ALDH2* gene is polymorphic and the variants demonstrate the vital role of ALDH2 in ethanol oxidation. Alcohol consumption causes facial flushing in a large proportion of Japanese, Chinese and Koreans (Wolff, 1972, 1973). The reaction has even been seen in Asian infants given alcohol, suggesting a genetic basis. Family studies suggested that the flush reaction is inherited as a dominant trait (Schwitters *et al.* 1982). The flushing reaction correlates with the accumulation of acetaldehyde (Zeiner *et al.* 1979; Goedde *et al.* 1983; Enomoto *et al.* 1991*a*). In non-flushers drinking alcohol elicits a small increase in acetaldehyde levels (to 5–10 μM); in flushers the levels are variable, but may exceed 100 μM (Enomoto *et al.* 1991*a*). The similarity between the Asian flush reaction and the disulfiram flush reaction (Asmussen *et al.* 1948) suggests that ALDH deficiency might be the explanation. A large percentage (about 40%) of Japanese have been found to lack ALDH2 activity in hair root and liver samples (Harada *et al.* 1981, 1982) and most of these individuals flushed when they drank alcohol. Thus, ALDH2 appears to play a crucial role in maintaining low levels of acetaldehyde during alcohol oxidation.

The mutation responsible for the deficiency is a G→A substitution that results in replacement of glutamate with lysine at position 487 in ALDH2 (Hempel *et al.* 1984; Yoshida *et al.* 1984). The normal allele is termed *ALDH2*1* and the mutant allele is designated *ALDH2*2*. *ALDH2*2* homozygotes have essentially no ALDH2 activity, while heterozygotes have markedly reduced but still detectable activity; hence, ALDH2 deficiency is a dominant negative trait (Crabb *et al.* 1989). Consistent with enzyme activity measurements, homozygotes experience far higher acetaldehyde levels after they drink alcohol than do heterozygotes (Enomoto *et al.* 1991*a*).

Measurement of ALDH activity in the livers of controls and individuals with ALDH2 deficiency suggested that about 40% of total liver ALDH activity is ALDH2 and 60% comprises other forms (ALDH1, ALDHE3 and possibly ALDH5; SJ Yin, personal communication). The *ALDH2*2* allele encodes an enzyme with a much increased K_m for NAD^+ and a reduced V_{max} when compared with the wild-type enzyme (Farres *et al*. 1994*b*). Thus, it is predicted to be virtually inactive under conditions occurring in liver mitochondria. The two *ALDH2* alleles have been expressed in tissue culture cells (Xiao *et al*. 1995). Transduction of *ALDH2*1* resulted in expression of a low-K_m ALDH with the expected isoelectric point. The *ALDH2*2* allele directed expression of an inactive protein. Transduction of *ALDH2*2* into *ALDH2*1* expressing cells reduced the low K_m activity substantially. The extent of reduction in activity suggested that only tetramers containing either three or four wild-type subunits are active. Moreover, the *ALDH2*2* polypeptides were less stable in the transduced cell lines, further reducing the level, and thus activity, of heterotetramers (Xiao *et al*. 1996) and contributing to the dominance of the *ALDH2*2* allele. The x-ray crystal structure of ALDH2 shows that amino acid 487 is situated in a region of the protein involved in subunit–subunit interactions (Steinmetz *et al*. 1997). Introduction of a positive charge by substitution of a lysine for glutamate disrupts essential ionic bonds and is predicted to inactivate the adjacent subunits and explain the dominance of the mutation.

Studies of the effect of ALDH2 deficiency on alcohol metabolism rates are limited by the adverse effects of the flush reaction. Preliminary studies failed to show a difference in alcohol elimination rates between flushers and non-flushers (Mizoi *et al*. 1979; Inoue *et al*. 1984), but a subsequent study detected reduced rates of elimination in individuals with ALDH2 deficiency when controlled for ADH genotype (Mizoi *et al*. 1994). This finding would be consistent with product inhibition of ADH by elevated intrahepatic acetaldehyde levels.

A polymorphism in the *ALDH2* promoter was simultaneously reported by Harada *et al*. (1999) and Chou *et al*. (1999). This A/G variant occurs at approximately −360 bp upstream from the start site and is adjacent to a site bound by transcription factors belonging to the steroid receptor family. The A allele is less active than the G allele in reporter gene transfection assays. Harada *et al*. (1999) showed that the A allele was also less common in a group of alcoholics with active ALDH2. These variants were found in all ethnic groups examined. It will be very interesting to see if the observations on the association of the A allele with protection from alcoholism can be extended to Caucasians and Africans.

Correlation between genetic variants and risk of alcoholism and organ-specific injury

The genetic predisposition to alcoholism has been amply demonstrated by a number of classical genetic studies, such as twin, adoption and high-risk familial clustering studies. Despite use of unbiased approaches such as genome-wide screening, the strongest genetic associations identified to date are those related to the *ADH* and *ALDH2* genes. Specifically, individuals having the genes encoding high-activity ADH (β2 ADH encoded by *ADH2*2*) or the dominant negative allele for ALDH2 (*ALDH2*2*) are at reduced risk of alcoholism, while those with *ALDH2*2* are at much higher risk of oro-pharyngeal cancer. Associations with other disorders are less strong at present. Most work has concentrated on an imbalance between the rate of acetaldehyde production and disposal as the likely explanation for associations between ADH and ALDH2 polymorphisms and various pathologies. This thinking is strongly influenced by the phenomenon of the alcohol flush reaction. However, additional mechanisms by which the inheritance of different isozymes alters risk for disease need to be considered in order to interpret the association studies. These mechanisms include: interference by ethanol in the metabolism of retinol or other metabolites by ADH or ALDH2 and effects of ethanol on redox state and the metabolism of compounds such as steroid hormones. In addition, oxidative stress induced by ethanol and effects of acetaldehyde on signalling pathways in various tissues may also prove to be important.

Alcohol dehydrogenase

Effects on risk of alcoholism. Despite the small effect of *ADH* genotype on alcohol elimination rate, *ADH* genotypes, particularly the presence of an *ADH2*2* allele, are related to differences in alcohol-drinking behaviour. Among Chinese living in Taiwan the *ADH2*2* allele was found to be substantially more common in the non-alcoholic group than in the alcoholics (Thomasson *et al*. 1991). Similar findings have been reported in the Atayal natives of Taiwan (Thomasson *et al*. 1994), the Maori of New Zealand (Chambers *et al*. 2002), in Spanish patients (Borras *et al*. 2000) and among Jews living in the USA or in Israel (Neumark *et al*. 1998). There was no apparent effect of *ADH2* alleles on the quantity and frequency of drinking in Japanese men (Takeshita *et al*. 1994), although the number of individuals with the genotypes expected to predispose to the highest consumption (individuals homozygous for both *ALDH2*1* and *ADH2*1*) is small because of the allele frequencies in this population. A more recent study indicated that *ADH2*1* was more common in heavy drinkers than in moderate drinkers (Tanaka *et al*. 1997). Moreover, the *ADH3*1* allele was also more prevalent in the non-alcoholics than in the alcoholics (Shen *et al*. 1997) in Asians, but there is no apparent effect of the *ADH3* locus on alcohol consumption or alcoholism rates in Caucasians (Gilder *et al*. 1993). The mechanism for this protective effect is uncertain. Since the *ADH2*2* allele encodes the highly-active β2 ADH isozyme, it has been postulated that faster conversion of alcohol to acetaldehyde could be 'protective' against heavy drinking and alcoholism. However, alcohol elimination rates and peak blood acetaldehyde levels were not influenced by the *ADH2*2* genotype (Mizoi *et al*. 1994).

Fewer studies on the relationship between the *ADH2*3* allele and risk of alcoholism or drinking behaviour have been carried out. The presence of *ADH2*3* was associated with a negative family history of alcoholism

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(Ehlers *et al*. 2001) and with greater alcohol expectancies (Ehlers *et al*. 2003). However, the *ADH2*3* allele was not found at a different frequency in alcoholics and controls in a study of >200 African-Americans (Taylor *et al*. 2003). On the other hand, the presence of *ADH2*3* was associated with a lower 'maximum number of drinks' among Mission Indians in California, USA (Wall *et al*. 2003). Additional work is needed to find out whether this allele modifies more characteristics associated with the subtle aspects of alcohol consumption.

Effect on risk of liver disease. The evidence for genetic risk factors for alcoholic liver disease is less strong than that for alcoholism. The largest study, the US Veterans Administration Twin Panel Study, showed a higher concordance for cirrhosis in monozygotic twins than in dizygotic twins, indicating a genetic component to the risk (Hrubec & Omenn, 1981). Re-analysis of the database supported this conclusion, but found that most, but not all, of the genetic liability for cirrhosis was the result of shared risk for alcoholism (Reed *et al*. 1996). No specific candidate genes that confer risk or protection against alcoholic liver disease have been firmly established, but there are hints that the ADH polymorphisms may play some role.

The effect of ADH variants on the risk of alcoholic liver disease could be complex (Lumeng & Crabb, 1994). High-activity ADH variants decrease alcoholism risk, but if individuals with these isozymes persist in drinking, hepatic injury might result from high intrahepatic concentrations of acetaldehyde. One study has demonstrated increased risk of alcoholic liver diseases among *ADH2*2* hetero- or homozygotes (Yamauchi *et al*. 1995), and pooling two other studies with this study (Chao *et al*. 1994; Tanaka *et al*. 1996), provided evidence of a substantial increase in risk of cirrhosis in the subjects with *ADH2*2*.

ADH3 is polymorphic in Caucasians. Two studies of the prevalence of *ADH3*1* and *ADH3*2*, in patients from the UK (Day *et al*. 1991) and from France (Poupon *et al*. 1992), have suggested that *ADH3*1*, encoding the more active enzyme, may be more common in those with alcoholic liver diseases. When the data from the two studies were pooled, the prevalence of *ADH3*1* was 0·65 in alcoholics with cirrhosis and 0·55 in controls (Day *et al*. 1993) and the difference approached significance. However, a lower *ADH3*1* allele frequency was reported in individuals with cirrhosis in another study (Sherman *et al*. 1994). Thus, the effect of this polymorphism is uncertain.

An additional ADH genetic variant, which occurs within an intron, is a *Pvu* II restriction fragment length polymorphism in the *ADH2* gene. It is not known whether the variant alters the expression of the gene or is linked to another susceptibility locus. The B allele was found at a considerably higher frequency in alcoholics and seemed to be more common in patients with cirrhosis rather than alcoholic hepatitis (Sherman *et al*. 1993).

Contributions of alcohol dehydrogenase to other disorders. Although the majority of ethanol is eliminated by hepatic oxidation, ADH is expressed in many tissues (Saleem *et al*. 1984; Engeland & Maret, 1993; Estonius *et al*. 1996), and there is the opportunity, therefore, for alcohol either to be metabolized to potentially-injurious acetaldehyde or to interfere with the metabolism of compounds normally oxidized by this enzyme. Retinoic acid, generated from retinol oxidation, plays an important role in the regulation of embryonic development, spermatogenesis and epithelial differentiation by serving as a ligand for members of the nuclear receptor family. Based on careful kinetic studies of all human ADH family members, ethanol at concentrations found in heavy drinkers can effectively block the oxidation of retinol to retinoic acid. Accordingly, Yin *et al*. (1999) further suggested that inhibition of retinol oxidation by ethanol might contribute to testicular atrophy, oligospermia, psoriasis and the increased incidence of oral, oesophageal and colo-rectal cancers in chronic alcoholics. Indeed, an association between *ADH2*1* and testicular atrophy in alcohol abusers has been reported (Yamauchi *et al*. 2001). Several studies have attempted to link *ADH* genotype with risk of fetal alcohol syndrome. One study found a protective effect of *ADH2*3* (McCarver *et al*. 1997), while another found it to be a risk factor (Stoler *et al*. 2002). Another study reported that *ADH2*2* is protective against fetal alcohol syndrome (Viljoen *et al*. 2001).

A small study indicated that *ADH2*2* may be more prevalent in individuals with alcoholic pancreatitis than in alcoholics who do not have this complication (Matsumoto *et al*. 1996). There may also be an association between the homozygous *ADH3*1* genotype and risk of oral cancer (Coutelle *et al*. 1997; Harty *et al*. 1997); however, this gene is not reported to be active in the oral mucosa (Dong *et al*. 1996). Epidemiological evidence points to an increased risk of rectal cancer in heavy drinkers. Seitz *et al*. (1996) have found ADH activity in the rectum that is similar in magnitude to that found in the stomach. Recently, it was reported that alcohol consumption increases the risk of adenomatous colon polyps to a greater extent in individuals homozygous for *ADH3*1* than in other genotypes (Tiemersma *et al*. 2003).

There has been much interest in the potential interaction between alcohol, *ADH* genotype and risk of breast cancer. Epidemiological studies suggest an increase in breast cancer among women who drink more than one or two standard drinks per d. ADH is highly expressed in the mammary epithelium, which lacks low-K_m ALDH; hence, there may be local metabolism of ethanol in the breast tissue (Triano *et al*. 2003). One study reported an increased risk of breast cancer among premenopausal, but not postmenopausal, women who were *ADH3*1* homozygotes (Freudenheim *et al*. 1999), while another study found no such association (Hines *et al*. 2000). The most recent study found an interaction between the *ADH2*2* allele in Caucasian women and alcohol consumption among patients with breast cancer (Sturmer *et al*. 2002). The *ADH2*2* allele was more common in the women with breast cancer. Among the women with breast cancer those with *ADH2*2* were less likely to drink more than once per week than those with *ADH2*1*.

Aldehyde dehydrogenase 2

Effects on risk of alcoholism. Japanese studies demonstrated that ALDH2 deficiency reduced the quantity and

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frequency of alcohol consumption by men and the risk of alcoholism (Higuchi *et al*. 1992). This effect was confirmed in other Asian populations by the observation that individuals who were alcoholic (Goedde *et al*. 1983; Harada *et al*. 1983, 1985) or who had alcoholic liver disease (Shibuya & Yoshida, 1989) rarely had ALDH2 deficiency or an *ALDH2*2* allele. In Japan about 41% of controls were ALDH2 deficient, while only 2–5% of alcoholics were ALDH2 deficient (Harada *et al*. 1983). In Taiwanese males the frequency of the *ALDH2*2* allele was 30% in a non-alcoholic control group and 6% in alcoholics (Thomasson *et al*. 1991). Similar results were reported by other research groups. The protective effect of being heterozygous for *ALDH2*2* appears to be decreasing over time in Japan (Higuchi *et al*. 1994), i.e. the frequency of *ALDH2*2* heterozygotes among alcoholics is increasing, presumably because of environmental and cultural changes. However, *ALDH2*2* homozygotes are nearly absolutely protected against alcoholism, presumably because of the severity of their flushing (Higuchi *et al*. 1994; Chao, 1995). This phenomenon has been observed in other countries (China and Pacific islands; Agarwal *et al*. 1981; Goedde *et al*. 1983, 1989; Goedde & Agarwal, 1987) with a somewhat different prevalence of the *ALDH2*2* allele, as well as in Asians living in Canada, suggesting that the flush reaction is protective against alcoholism for biochemical rather than cultural reasons (Tu & Israel, 1995).

Effect on risk of liver disease. ALDH2 deficiency may be a two-edged sword for the reasons mentioned earlier for *ADH2*2*, since individuals with mild flushing who can tolerate heavy drinking may suffer from the hepatic effects of elevated acetaldehyde concentrations. There has been one small study suggesting that *ALDH2*2* heterozygotes who drink heavily develop alcoholic hepatitis at a lower cumulative alcohol consumption than those with active ALDH2 (Enomoto *et al*. 1991*b*). When the results of several studies were combined the prevalence of *ALDH2*2* was substantially higher in the alcoholic patients with cirrhosis than in those without cirrhosis (Chao *et al*. 1994; Yamauchi *et al*. 1995; Tanaka *et al*. 1996). With the apparent increase in the number of alcoholics heterozygous for *ALDH2*2* in Japan, ALDH2 deficiency may become an important risk factor in that population.

Effects on other health risks. ALDH2 deficiency is associated with alcohol-induced asthma, thought to result from the effect of increased circulating or locally-generated acetaldehyde on the airways (Takada *et al*. 1994). Several groups have reported that ALDH2 deficiency is associated with increased risk of oesophageal and oropharyngeal cancer (Yokoyama *et al*. 1996*a*,*b*,*c*). The activity of ADH is considerably higher than that of ALDH in the oesophagus, which would predispose this tissue to injury during ethanol oxidation (Yin *et al*. 1993, 1997). It is also possible that the phenomenon reflects increased exposure of the oropharynx and oesophagus to acetaldehyde in the saliva (Vakevainen *et al*. 2000, 2001). The amount of acetaldehyde in saliva is increased in *ALDH2*2* heterozygotes given alcohol, and this amount falls when alcohol oxidation is inhibited by 4-methylpyrazole, suggesting that the acetaldehyde is generated in the salivary glands. An additional observation is the association between ALDH2 deficiency and a mitochondrial DNA mutation and diabetes in Japanese subjects. It was hypothesized that ALDH2 deficiency predisposed the patients to mutagenic effects of acetaldehyde on mitochondrial DNA (Suzuki *et al*. 1996). No effect of ALDH2 deficiency on the risk of stomach cancer or hepatoma has been reported.

Summary

Many lines of evidence indicate that the genetic variation in ADH and ALDH2 contribute to the risk of alcoholism and the susceptibility to certain alcohol-induced pathologies. Most of the evidence has come from association studies, which of course do not prove a causative relationship. The next challenge is to confirm the findings and to understand the biochemical mechanisms responsible for the risk and susceptibility, which will point toward a better understanding overall of the disease processes.

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