Chapter 2
Genetics of Alcohol Metabolism

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Key Points

- Alcohol metabolism occurs mainly via hepatic oxidation and is governed by the catalytic properties
  of the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH), and aldehyde dehydrogenase
  (ALDH2).
- Genetic polymorphisms in ADH1B and ALDH2, and ethnic differences in the prevalence of these
  polymorphisms, result in increased variation in alcohol metabolism among individuals.
- Polymorphisms in ADH1B result in variants that code for isozymes that tend to show a faster rate
  of alcohol metabolism, while the ALDH2*2 polymorphism results in a “deficient” form of ALDH2
  that causes an accumulation of acetaldehyde and its associated physiological effects.
- ADH and ALDH polymorphisms are also associated with a protective effect on the development
  of alcoholism. The allele frequencies of ADH1B*2, ADH1B*3, and ALDH2*2 are significantly lower
  in individuals diagnosed with alcohol dependence compared to controls.
- Further evaluation of the factors, both genetic and environmental, regulating the rates of alcohol
  and acetaldehyde metabolism, will help improve our understanding of the metabolic basis and
  consequences of alcohol’s effects, including the risk and consequences of alcohol-related organ
  damage, developmental problems, as well as alcohol dependence.

Keywords  Alcohol metabolism • Alcohol dehydrogenase (ADH) • Aldehyde dehydrogenase (ALDH)
• Genetic polymorphism • Ethnic differences • Cytochrome P450 • Catalase • Pharmacogenetics

Introduction

Ethanol (also referred to as alcohol in this chapter) is probably the most widely investigated drug in
the world, not only because of its ubiquitous use and its widespread abuse but also because of its
unique pharmacological properties. Following administration, systemic concentrations of alcohol are
a consequence of the absorption, distribution, and metabolism of alcohol, which display very unique
characteristics and demonstrate substantial interindividual variability [1]. As the pharmacological
effects of alcohol depend on its systemic concentrations, variability in the pharmacokinetics of alcohol can have a significant impact on its pharmacodynamic effects.

Following oral ingestion, alcohol is absorbed by passive diffusion, primarily from the small intestine [2, 3]. The rate of absorption depends on several factors, both genetic and environmental, and is highly variable. Some of these factors include the volume, concentration, and nature of the alcoholic beverage [2, 4, 5]; the rate of drinking [4]; the fed or fasted state [6]; the nature and composition of food [6, 7]; the rate of gastric emptying [8, 9]; the gender differences in first-pass metabolism [10, 11]; and other drugs including histamine (H1) receptor antagonists like cimetidine and ranitidine [12, 13]. Ethanol is a small polar molecule and its volume of distribution is comparable to total body water [3]. No plasma protein binding has been reported for alcohol. Elimination of alcohol occurs primarily through metabolism with small fractions of the administered dose being excreted in the breath (0.7%), sweat (0.1%), and urine (0.3%) [3]. Alcohol metabolism occurs mainly via hepatic oxidation and is governed by the catalytic properties of the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). The cytochrome P450 enzymes (CYP2E1) and catalase also contribute to alcohol metabolism and alcohol-related cytotoxicity under specific circumstances [14].

Alcohol metabolic rates show a considerable degree of interindividual and ethnic variability, in part due to allelic variants of the genes encoding ADH and ALDH producing functionally different isozymes [15–17]. Functional polymorphisms of the ADH1B and ALDH2 genes have been shown to increase the variance in alcohol metabolism among individuals. Additionally, a multitude of environmental factors can influence the metabolic regulation of alcohol metabolism, which results in a large three- to four-fold variance in the alcohol elimination rate in humans [16, 18]. Factors that have been shown to be important determinants of alcohol metabolism include age [19, 20], gender [21, 22], ethnicity and genetics [21, 23–26], body mass and liver size [22], as well as environmental factors such as food intake [27].

This chapter will focus on genetic variation in the alcohol-metabolizing enzymes and its impact on the metabolism of alcohol.

**Alcohol-Metabolizing Enzymes and Genetic Aspects**

**Alcohol Dehydrogenase**

The genes for the human ADH family cluster in a region of chromosome 4q21 spanning ~370 kb [28]. The alcohol dehydrogenase (ADH) gene family encodes oxidative enzymes that metabolize a wide variety of alcohols including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products [15, 17]. Currently, seven human ADH genes have been identified and organized into five classes based on amino acid sequence alignments, catalytic properties, and patterns of tissue-specific expression [29]. Human ADH enzyme is a dimeric molecule, arising from the association of different subunits expressed by the seven genes. Thus, there are over 20 ADH isozymes that vary greatly with regard to the types of alcohols they preferentially metabolize and the maximal rate at which they oxidize ethanol [15]. The five classes of ADH are divided according to their subunit and isoenzyme composition (Table 2.1).

The class I isozymes are found in liver and consist of homo- and heterodimeric forms of the three subunits (i.e., αα, αβ, ββ, βγ, γγ, etc.). Classes II, III, and IV enzymes are homodimeric forms of the π, χ, and σ subunits, respectively. All the class I ADHs metabolize ethanol and are inhibited by pyrazole derivatives [17]. The ADH1B subunits share about 94% sequence identity. The relative order of catalytic efficiency (kcat/Km) for ethanol oxidation at ethanol concentrations of about 100 mg% and saturating coenzyme NAD+ concentration (0.5 mM) is β2 > β1 > γ1 > γ2 ≈ σ > β3 > α ≈ π. However, the relative order of kcat at saturating concentrations of both ethanol and NAD+ is σ > β3 > β2 > γ1 > γ2 ≈ π > β1. Thus, the relative contributions of each of the ADH isozymes to ethanol oxidation change with the hepatic concentration of alcohol [16, 17, 30].
The human ADH genes are differentially expressed in different tissues, and this is a fundamental determinant of the physiological consequences of alcohol metabolism in specific cells and tissues [31]. The liver contains a large amount of ADH (about 3% of soluble protein) and expresses the widest number of different isozymes. ADH4 (p-ADH) is solely expressed in liver. Only ADH7 (s-ADH) is not highly expressed in liver. ADH5 (c-ADH) is ubiquitously expressed in human tissues. ADH1C, ADH4, ADH5, and ADH7 are expressed in gastrointestinal tissues. The expression of ADH6 in humans and its role in ethanol metabolism remains to be elucidated. The expression of ADH in other tissues such as skeletal muscle, and the quantitative significance of muscle ADH metabolism (because of the large proportion of muscle mass in the body), also remains to be determined.

In addition to ethanol, alcohol dehydrogenases also oxidize several “physiological” alcohols with high catalytic efficiency including retinol, α-hydroxy fatty acids, hydroxysteroids, and hydroxy derivatives of dopamine and epinephrine metabolites [30, 32]. Oxidation of these alcohols can be inhibited by ethanol, and therefore the role of ethanol substrate competition is an important issue in alcohol-related toxicology. Another important issue is the regional expression of ADHs in brain and their potential role in the local formation of acetaldehyde, which may be centrally active, possessing stimulant as well as sedative/hypnotic effects [33–35].

### Genetic Variation

Single nucleotide polymorphisms (SNP) have been identified at the ADH1B and ADH1C loci [15, 17, 31]. Variant alleles of ADH1B result in the β1, β2, and β3 subunits, while variants in ADH1C result in the γ1 and γ2 subunits. The resulting subunits have different catalytic activities for ethanol (see Table 2.1). Additionally, the ADH1B alleles appear with different frequencies in different racial groups, with the ADH1B*1 form predominating in Caucasian and African-descent populations, and ADH1C*2 predominating in East Asian populations (e.g., Chinese and Japanese), and also found in about 25% of Caucasians with Jewish ancestry. The ADH1B*3 form is found in about 25% of individuals of African descent. With respect to the ADH1C polymorphism, ADH1C*1 and ADH1C*2 appear with about equal frequency in Caucasians, but ADH1C*1 predominates in African-descent and East Asian populations [36]. Recently, a novel polymorphism was identified in ADH1C. This polymorphism results in an allele that codes for a subunit with a proline to threonine substitution in position 351 and has been described in Native Americans [37]. However, the catalytic activity of the isozyme coded by this variant and its effect on the overall elimination of alcohol remains to be determined.

There are additional SNPs that have been identified in the noncoding regions of the ADH genes. Several of these SNPs have been shown to affect the expression of ADH genes [31, 38] and may be
associated with alcoholism risk [39]; however, the effect of these variations on the catalytic activity of ADH and effect on the overall metabolism of alcohol remains to be established.

**Aldehyde Dehydrogenase**

Acetaldehyde is the first metabolic product of ethanol metabolism and is itself metabolized via oxidation by the NAD+-dependent aldehyde dehydrogenase (ALDH). Several isozymes of ALDH, differing in kinetic properties and tissue distribution, have been detected in human organs and tissues [15]. Currently, 19 putatively functional ALDH genes have been identified in the human genome [40, 41]. However, only the ALDH1 (ALDH1A1) and ALDH2 genes encode the class I and class II isozymes that are involved in acetaldehyde oxidation. ALDH1 is the cytosolic form distributed ubiquitously in tissues including brain. It exhibits relatively low catalytic activity (Km ~ 30 μM) for acetaldehyde oxidation. ALDH2 is the mitochondrial enzyme that is highly expressed in liver and stomach [42]. It exhibits high catalytic activity (Km ~ 3 μM) for acetaldehyde oxidation and is primarily responsible for acetaldehyde oxidation in vivo.

**Genetic Variation**

The best-known genetic polymorphism in ALDH genes is in ALDH2. The allelic variants are ALDH2*1 and ALDH2*2, encoding for the high-activity and low-activity forms of the subunits respectively. The low-activity form arises from a single amino acid exchange (glutamine to lysine substitution at position 487) at the coenzyme-binding site of the enzyme subunit [15, 17]. This results in a 100-fold increase in the Km for acetaldehyde [43]. This very prominent variant allele has been seen in about half of the East Asian populations studied (including the Han Chinese, Taiwanese, and Japanese) [44, 45]. It has not been observed in populations of Caucasian origin. It exhibits virtually no acetaldehyde oxidizing activity in vitro and represents the “deficient” phenotype seen in these Asian populations [46]. Individuals who are heterozygous or homozygous for ALDH2*2 show accumulation of acetaldehyde levels and the characteristic sensitivity reaction (facial flushing, increased skin temperature and heart rate) following alcohol intake [26, 28, 47, 48].

**Cytochrome P450 Enzymes**

A small fraction of an ingested dose of ethanol is metabolized by enzymes other than ADH. Metabolism of ethanol by the so-called microsomal ethanol oxidizing system (MEOS) accounts for the major non-ADH system [14, 49]. MEOS consists primarily of the cytochrome P450 isoform, P4502E1 (CYP2E1), along with other P450 enzymes, and is the major alternative system that catalyzes the NADPH- and O2-dependent oxidation of ethanol to form acetaldehyde, NADP+, and water. Like other cytochrome P450 enzymes, the primary role of CYP3E1 is the metabolism of alcohol and other xenobiotics. While CYP2E1 accounts for a much smaller fraction of ethanol oxidation than the ADH system under normal conditions, it represents a major adaptive response of alcohol metabolism with chronic ethanol consumption [49]. This is due to the direct effect of chronic ethanol consumption on the expression of hepatic CYP2E1. In humans, there is an induction of CYP2E1 with chronic alcohol consumption that can be followed by a decrease in activity associated with generalized hepatic injury and loss of function. There are two mechanisms postulated for CYP2E1 induction: (1) a posttranslational mechanism involving mRNA stabilization and protection of the expressed protein against degradation and (2) a
direct transcriptional regulation of \textit{CYP2E1} expression, generally following high exposures to ethanol. The expression of \textit{CYP2E1} is influenced by factors such as diet (lipids, carbohydrates) and hormones (thyroid hormones, glucocorticoids, steroids, pituitary hormones). The induction of \textit{CYP2E1} may result in higher levels of toxic metabolites of other xenobiotics as well as the generation of superoxide radicals, which may contribute to the increased risk of alcohol-related liver disease as well as cancer.

**Genetic Variation**

A number of different \textit{CYP2E1} polymorphisms have been identified [15, 50]. A variant allele called *5B has been identified in the 5'-flanking region of the \textit{CYP2E1} gene. This allele has been shown to be differentially expressed in different racial populations, and the variant allele (previously labeled as the \textit{c2} allele) has been found to be associated with higher transcriptional activity, protein levels, and enzyme activity than the common wild-type \textit{c1} allele [51]. The influence of this polymorphism on alcohol elimination was examined in one study in Japanese alcoholics and control and indicated that the presence of the \textit{c2} allele (heterozygous or homozygous) may be associated with higher alcohol metabolic rates but only at blood alcohol levels greater than 0.25% (g/dL) [52]. Studies have identified additional genetic variation that may be relevant to alcohol, including the *1D allele, which has been found at higher frequency in Chinese (23%) and African-Americans (31%) than in Caucasians (1–7%) [53, 54]. Studies in African-Americans have further shown higher levels of \textit{CYP2E1} inducibility following alcohol intake as measured by oxidation of the \textit{CYP2E1} substrate chlorzoxazone. However, the influence of this polymorphism on alcohol metabolism remains to be determined. Much work needs to be done to understand mechanisms for transcriptional and posttranslational regulation of the \textit{CYP2E1} genes and their role in alcohol metabolism and alcohol-related liver disease [49].

**Catalase**

Catalase is an enzyme that catalyzes the hydrogen peroxide (H$_2$O$_2$)-dependent oxidation of ethanol yielding acetaldehyde and two molecules of water. It is found in the cytosol and mitochondria but its main expression and function is in peroxisomes. Most studies indicate that it contributes very little to total ethanol elimination because of the limited availability of hydrogen peroxide [14, 55]. However, the activation of peroxisomal catalase by increased generation of hydrogen peroxide via peroxisomal \(\beta\)-oxidation can lead to a hypermetabolic state and a swift increase in alcohol metabolism under some conditions [56]. This state may contribute to alcohol-related inflammation and necrosis in alcoholic liver disease. Additional studies have suggested that catalase may be involved in the metabolism of alcohol to acetaldehyde in the brain. This has led to implications of a role for acetaldehyde in mediating some of the behavioral effects of alcohol [35]. However, further research is needed to clarify the pharmacokinetics and central pharmacodynamic effects of acetaldehyde and its role in the pharmacology of alcohol.

**ADH and ALDH Polymorphisms: Influence on Alcohol Metabolism**

Functional polymorphisms of genes for the alcohol-metabolizing enzymes \textit{ADH} and \textit{ALDH2}, and differences in the prevalence of the polymorphic alleles in different ethnic populations, have resulted in several studies examining ethnic differences in alcohol metabolism and the influence of \textit{ADH1B}, \textit{ADH1C}, and \textit{ALDH2} genotypes. The isozymes encoded by the polymorphic alleles have very different catalytic properties in vitro, as described earlier in this chapter, and would be expected to exert influences on an individual’s alcohol metabolic rate.
One of the first studies examining the influence of ADH and ALDH polymorphisms on alcohol metabolism was done by Mizoi et al. [23] in 68 healthy Japanese subjects. Subjects were genotyped for ADH1B as well as ALDH2 polymorphisms and alcohol disappearance rates (mg/ml/h), and elimination rates (mg/kg/h) were compared among the groups classified based on genotypes of both ADH1B (ADH1B*1/*1, ADH1B*1/*2, and ADH1B*2/*2) and ALDH2. Results indicated that there were no differences in alcohol metabolism among the ADH1B genotypes; however, there were marked differences among the ALDH2 genotypes with regard to alcohol metabolism. Other studies in Asians have also failed to demonstrate an effect of the ADH1B*2 allele on alcohol metabolism after controlling for the ALDH2*2 polymorphism. This is discussed further below.

Studies in Jewish individuals possessing the ADH1B*2 polymorphism have provided a clearer picture of the effect of this variant on alcohol metabolism, Neumark et al. [57] conducted a study in young healthy Jewish males to assess the effect of the ADH1B polymorphism on alcohol elimination rates measured using the alcohol clamp [58]. Results revealed a significantly higher alcohol elimination rates in subjects carrying the ADH1B*2 allele (heterozygotes and homozygotes) compared with ADH1B*1 homozygotes [57, 59]. As the Jewish do not show polymorphisms of the ALDH2 genes, this appears to be a direct effect of ADH1B genotypes on alcohol metabolism.

Thomasson et al. [21] examined the influence of the ADH1B*3 polymorphism on alcohol metabolism in a sample of 112 African-American subjects, selected by genotype. In this study, subjects received an oral dose of alcohol and alcohol disappearance rates were determined from the slope of the pseudo-linear portion of the blood ethanol concentration vs. time curves. Results revealed that subjects carrying the ADH1B*3 allele (heterozygotes and homozygotes) showed a higher alcohol disappearance rate (mg% per h) for compared to ADH1B*1 homozygotes. A more recent study in African-Americans failed to demonstrate an effect of the ADH1B*3 polymorphism on breath alcohol concentrations following a moderate oral dose of alcohol in 91 African-Americans [60]. A study in Native Americans also showed that subjects with ADH1B*3 alleles had a trend toward higher alcohol elimination rates than subjects with ADH1B*1 [24]. However, this difference was not statistically significant probably because of the small number of subjects possessing the ADH1B*3 genotype in the study and the low frequency of occurrence of this genotype (~7%) in this ethnic group. Earlier studies in Native Americans have previously demonstrated higher alcohol elimination rates compared to those reported in Caucasians; however, ADH genotypes were not determined in these studies [61, 62].

The influence of ALDH2 polymorphisms on alcohol metabolism has been studied more extensively, although almost exclusively in Asian subjects, mainly because of the high frequency of the polymorphism in this population. Most of these studies have compared peak concentrations of alcohol and acetaldehyde as well as peak responses on subjective and cardiovascular measures and flushing across ADH1B and ALDH2 genotypes, with generally consistent results. In general, individuals who are heterozygous or homozygous for ALDH2*2 show increased acetaldehyde levels following alcohol administration [23, 25, 28, 47, 63–65]. Some studies have also demonstrated significant increases in ethanol concentrations and area under the ethanol concentration time curves [63, 65], possibly due to product inhibition of the ADH activity by acetaldehyde. However, other studies have shown accumulation of acetaldehyde in subjects carrying the ALDH2*2 allele without any difference in alcohol concentrations or elimination rates [25, 26].

Given the high frequency of the ADH1B*2 and ALDH2*2 alleles in Asians, it is important to understand the contribution of each polymorphism to the observed differences in blood alcohol and acetaldehyde levels following alcohol administration. There are only a few studies that have actually estimated and compared alcohol disappearance rates or elimination rates among ADH1B and/or ALDH2 genotypes. In the study by Mizoi et al. [23] described above, peak acetaldehyde levels, alcohol disappearance rates (mg/ml/h), and elimination rates (mg/kg/h) were compared among subjects classified into groups based on genotypes of both ADH1B and ALDH2 (ALDH2*1/*1, ALDH2*1/*2, and ALDH2*2/*2). Results indicated that subjects homozygous for ALDH2*1/*1 showed no increase in acetaldehyde levels regardless of their ADH1B genotype. There was a progressive increase in peak acetaldehyde levels in subjects with the ALDH2*1/*2 and ALDH2*2/*2 genotypes. Both alcohol
disappearance rates and elimination rates showed significant differences among the \( ALDH2 \) genotypes and decreased in the following order: \( ALDH2^{*1/*1} > ALDH2^{*1/*2} > ALDH2^{*2/*2} \). A study in Chinese men indicated that the presence of the \( ALDH2^{*2} \) allele was associated with slower alcohol metabolism following oral administration, while in individuals homozygous for \( ALDH2^{*1} \), the presence of two \( ADH2^{*2} \) alleles correlated with slightly faster alcohol metabolism [66]. Studies by Peng et al. [26, 48, 63] have demonstrated a clear effect of \( ALDH2 \) genotype on alcohol and acetaldehyde metabolism, as well as the lack of significant effect of \( ADH1B \) polymorphism on acetaldehyde metabolism. In fact, most studies in Asians have not demonstrated that the \( ADH1B^{*2} \) allele is associated with differences in alcohol metabolism after controlling for the \( ALDH2 \) [25, 47, 67].

A recent effort in understanding the influence of genetic variation in alcohol-metabolizing enzymes on alcohol metabolism has focused on the use of association analysis in a large cohort of twin pairs of Caucasian ancestry. In these studies, 103 SNPs spanning the \( ADH \) gene family were examined for association with measures of alcohol metabolism following oral alcohol challenge in this sample. Results indicated significant associations between alcohol elimination rates and \( ADH1A, ADH1B, ADH1C \), as well as \( ADH7 \) genes [68, 69]. These studies point to a role for \( ADH7 \) in the metabolism of alcohol; however, more work is needed to clarify the influence of this isoform, and its associated genetic variation, on alcohol elimination rates in humans.

In summary, genetic polymorphisms of \( ADH \) and \( ALDH \) result in alterations in the metabolism of alcohol and/or acetaldehyde. Polymorphisms in \( ADH1B \) result in variants that code for isozymes that tend to show a faster rate of alcohol metabolism, while the \( ALDH2^{*2} \) polymorphism results in a “deficient” form of \( ALDH2 \) that causes an accumulation of acetaldehyde and its associated physiological effects.

**ADH and ALDH Polymorphisms: Association with Alcohol Dependence**

Functional polymorphisms of the alcohol-metabolizing enzymes \( ADH \) and \( ALDH2 \) can also exert important effects on the biological effects of alcohol [26, 70]. In fact, the \( ADH \) and \( ALDH \) genes are the only genes which have been firmly established to influence vulnerability to alcohol dependence or alcoholism [17, 36]. Studies have demonstrated unequivocally that the allele frequencies of \( ADH1B^{*2}, ADH1B^{*3}, \) and \( ALDH2^{*2} \) are significantly decreased in subjects diagnosed with alcohol dependence as compared with the general population of East Asians, including the Japanese, Han Chinese, and Koreans [39, 44, 45, 67, 71–76]. The \( ALDH2^{*2} \) allele and the \( ADH1B^{*2} \) allele also significantly influence drinking behavior in nonalcoholic individuals. Association between reduced alcohol consumption or reduced risk of alcohol dependence and the \( ADH1B^{*2} \) variant allele has recently been found in other ethnic groups that do not carry the \( ALDH2^{*2} \) allele, including Europeans [77–80], Jews in Israel [81, 82], as well as Mongolians in China [45], and the Atayal natives of Taiwan [83]. Recent studies have also shown a protective association between the \( ADH1B^{*3} \) allele and alcohol dependence in Native Americans. [84, 85] Finally, studies have indicated that the \( ADH1B^{*3} \) allele may be protective against alcohol-related problems in infants born to African-American mothers who may have consumed alcohol during pregnancy [86–89].

**Summary**

There has been substantial progress in the field of alcohol pharmacogenetics to characterize differences in alcohol metabolism in subjects exhibiting polymorphic genotypes of the alcohol-metabolizing enzymes. The impact of functional variation in \( ADH1B \) and \( ALDH2 \) genes on alcohol metabolism have been fairly well characterized; however, there are large interindividual differences in alcohol elimination
rates that still remain unexplained. Of potential significance in this regard may be polymorphisms in ADH4 [90, 91], ADH7 [39, 69], and ALDH1A1 [92, 93] as well as the promoter regions of ALDH2 [94]. Further studies are needed to evaluate the influence of these polymorphisms on the activity of ADH and ALDH and on alcohol levels and elimination rates in individuals, as well as on the physiological response to alcohol consumption and alcoholism. Recent integrated approaches examining the associations of ADH and ALDH2 gene variation with alcohol metabolism, response, drinking behavior, and alcohol dependence in large samples [78] might be particularly useful in this regard.

Studies in monozygotic and dizygotic twins have shown that the heritability (i.e., genetic component of variance) of alcohol metabolic rates is about 50% [95, 96]. Further evaluation of the factors, both genetic and environmental, regulating the rates of alcohol and acetaldehyde metabolism, will help improve our understanding of the metabolic basis and consequences of alcohol’s effects, including the risk and consequences of alcohol-related organ damage, developmental problems, as well as alcohol dependence.

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