

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

Gia-Sheun Peng<sup>1</sup> and Shih-Jiun Yin<sup>2\*</sup>

<sup>1</sup>Department of Neurology, Tri-Service General Hospital, 325 Chenggong Road Section 2, Taipei 114, Taiwan

<sup>2</sup>Department of Biochemistry, National Defense Medical Center, 161 Minchuan East Road Section 6, Taipei 114, Taiwan

\*Correspondence to: Tel: +886 2 8792 3100 ext 18800; Fax: +886 2 8792 4818; E-mail: yinsj@ndmc.idv.tw

Date received (in revised form): 30th July 2008

## 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.<sup>1</sup> 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.<sup>2–4</sup> Alcoholism is a multifactorial, polygenic behavioural disorder involving complex gene–gene and gene–environment interactions.<sup>5,6</sup> 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.<sup>7</sup> 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.<sup>2,8</sup> 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.<sup>11</sup> Distribution of the functional polymorphisms of *ADH1B* and *ADH1C* genes show ethnic distinctions.<sup>2,12,13</sup> 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.<sup>14,15</sup> 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.<sup>16</sup> There are two ALDH2 allozymes — ALDH2E (also designated E<sub>4</sub>, where E stands for normal type subunit Glu487) and ALDH2K (K<sub>4</sub>, 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.<sup>17,18</sup> 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<sub>4</sub>, E<sub>3</sub>K and E<sub>2</sub>K<sub>2</sub> 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     | <i>ADH1A</i>   |                 | $\alpha\alpha$         | 5.2        | 0.24             | 0.047                   |
|        |       | <i>ADH1B</i>   | <i>ADH1B*1</i>  | $\beta_1\beta_1$       | 0.016      | 0.18             | 12                      |
|        |       |                | <i>ADH1B*2</i>  | $\beta_2\beta_2$       | 1.9        | 4.8              | 2.6                     |
|        |       |                | <i>ADH1B*3</i>  | $\beta_3\beta_3$       | 53         | 3.2              | 0.060                   |
|        |       | <i>ADH1C</i>   | <i>ADH1C*1</i>  | $\gamma_1\gamma_1$     | 0.11       | 0.81             |                         |
|        |       |                | <i>ADH1C*2</i>  | $\gamma_2\gamma_2$     | 0.061      | 0.47             |                         |
|        |       | II             | <i>ADH2</i>     |                        | $\pi\pi$   | 23               | 0.30                    |
|        | III   | <i>ADH3</i>    |                 | $\chi\chi$             | 3,400      | 0.83             |                         |
|        | IV    | <i>ADH4</i>    |                 | $\mu\mu(\sigma\sigma)$ | 58         | 11               | 0.19                    |
| ALDH   | I     | <i>ALDH1A1</i> |                 | Tetramer               | 0.033      | 0.63             | 19                      |
|        | II    | <i>ALDH2</i>   | <i>ALDH2*1</i>  | Tetramer( $E_4$ )      | 0.00020    | 0.60             | 3,000                   |
|        |       |                | <i>ALDH2*2</i>  | 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,<sup>1</sup> Yin et al.<sup>9</sup> and Lee et al.<sup>10</sup>

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.<sup>3,12</sup> *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*.<sup>1–3</sup> Homozygosity of variant allele *ADH1B\*2* *per se* can reduce the risk of alcohol dependence by eight-fold in East Asians,<sup>19</sup> whereas *ALDH2\*2* homozygosity appears to be almost completely protective against the disease.<sup>20–22</sup> Multiple logistic regression analyses

suggest that *ADH1B\*2* and *ALDH2\*2* may independently influence vulnerability to alcoholism.<sup>19,21,22</sup> 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.<sup>23,24</sup> 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.<sup>19,25</sup> 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.<sup>23,26,27</sup> 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.<sup>28</sup> Numerous SNPs have been identified in the genes of human *ALDH1A1* and *ALDH2*.<sup>15</sup> Thus far, *ALDH2\*2* is the only variant that has been proven to be causally associated with alcoholism.<sup>3,29–31</sup>

## Pharmacogenetics of ethanol metabolism

The liver is the major organ for ethanol metabolism.<sup>1,16,32</sup> Acetaldehyde concentrations in hepatocytes depend on both the rate of generation (ie ethanol oxidation by *ADH*s) and the rate of removal (ie acetaldehyde oxidation by *ALDH*s). 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 *ADH*s, 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.<sup>10,33</sup> At low ethanol levels, *ADH1B* (except for *1B3*) and *ADH1C* isozymes/allozymes are active in the liver owing to the low  $K_m$ s (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  $\mu\text{M}$ ) (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  $\mu\text{M}$ ) is comparable with peak acetaldehyde levels ( $\sim 15 \mu\text{M}$ )

in hepatic venous blood after ingestion of 0.8 g/kg ethanol.<sup>34</sup> Gastric first-pass metabolism can influence the bioavailability of ethanol.<sup>32</sup> *ADH1C*, *ADH3* and *ADH4* are expressed in the human stomach and may contribute to ethanol oxidation with quantitative significance.<sup>10,33,35</sup>

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.<sup>21,29</sup> The increased ethanol oxidation rate at higher alcohol concentrations can be attributed to the contributions of hepatic high- $K_m$  *ADH1A* and *ADH2*.<sup>10,33</sup> 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 ( $17 \pm 3 \mu\text{M}$ ; mean  $\pm$  SE) similar to that of the peak concentrations ( $24 \pm 1 \mu\text{M}$ ) of the heterozygotes.<sup>29</sup> 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  $\mu\text{M}$ ) 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 (n) | Mean peak acetaldehyde ( $\mu$ M) | Author                     |
|---------------------|------------------------|--------------|--------------------|-----------------------------------|----------------------------|
|                     | <i>ALDH2</i>           | <i>ADH1B</i> |                    |                                   |                            |
| 0.2                 | *1/*1                  | *2/*2        | 6                  | 1.0                               | Peng et al. <sup>29</sup>  |
| 0.2                 | *1/*2                  | *2/*2        | 6                  | 24                                | Peng et al. <sup>29</sup>  |
| 0.2                 | *2/*2                  | *2/*2        | 6                  | 75                                | Peng et al. <sup>29</sup>  |
| 0.3                 | *1/*1                  | *1/*1        | 6                  | 0.3                               | Peng et al. <sup>30</sup>  |
| 0.3                 | *1/*1                  | *2/*2        | 6                  | 0.2                               | Peng et al. <sup>30</sup>  |
| 0.3                 | *1/*2                  | *1/*1        | 6                  | 60                                | Peng et al. <sup>30</sup>  |
| 0.3                 | *1/*2                  | *2/*2        | 6                  | 60                                | Peng et al. <sup>30</sup>  |
| 0.4                 | *1/*1                  | *1/*1        | 5                  | 3.3                               | Mizoi et al. <sup>36</sup> |
| 0.4                 | *1/*1                  | *1/*2        | 8                  | 4.8                               | Mizoi et al. <sup>36</sup> |
| 0.4                 | *1/*1                  | *2/*2        | 20                 | 4.2                               | Mizoi et al. <sup>36</sup> |
| 0.4                 | *1/*2                  | *1/*1        | 4                  | 23                                | Mizoi et al. <sup>36</sup> |
| 0.4                 | *1/*2                  | *1/*2        | 9                  | 23                                | Mizoi et al. <sup>36</sup> |
| 0.4                 | *1/*2                  | *2/*2        | 16                 | 24                                | Mizoi et al. <sup>36</sup> |
| 0.4                 | *2/*2                  | *2/*2        | 6                  | 79                                | Mizoi et al. <sup>36</sup> |
| 0.5                 | *1/*1                  | *1/*1        | 8                  | 3.3                               | Peng et al. <sup>31</sup>  |
| 0.5                 | *1/*1                  | *2/*2        | 8                  | 4.0                               | Peng et al. <sup>31</sup>  |
| 0.5                 | *1/*2                  | *2/*2        | 16                 | 76                                | Peng et al. <sup>31</sup>  |
| 0.5                 | *2/*2                  | *2/*2        | 1 <sup>a</sup>     | 125                               | Chen et al. <sup>21</sup>  |

<sup>a</sup>An alcohol-dependent patient with normal liver function.

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase

homozygotes (75  $\mu$ M) receiving 0.2 g/kg ethanol.<sup>29,31</sup> 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.<sup>29–31,36,37</sup> 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,<sup>10,38</sup> 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- $K_m$  *ALDH*s. 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.

## Acknowledgments

The work in the authors' laboratory was supported by grants 89-2314-B016-28, 92-2314-B016-73 and 96-2320-B016-18-MY3 from the National Science Council, and EX91-8939BP from the National Health Research Institutes, Republic of China.

## References

1. Yin, S.-J. and Agarwal, D.P. (2001), 'Functional polymorphism of alcohol and aldehyde dehydrogenases: Alcohol metabolism, alcoholism, and alcohol-induced organ damage', in: Agarwal, D.P. and Seitz, H.K. (eds), *Alcohol in Health and Disease*, Marcel Dekker, New York, NY, pp. 1–26.
2. Yin, S.-J., Lee, S.-L., Han, C.-L. *et al.* (2006), 'Pharmacogenetic determinants of alcohol metabolism and alcoholism in the human alcohol dehydrogenase family', in: Weiner, H., Lindahl, R., Plapp, B.V. *et al.* (eds), *Enzymology and Molecular Biology of Carbonyl Metabolism 12*, Purdue University, West Lafayette, IN, pp. 161–170.
3. Yin, S.-J. and Peng, G.-S. (2005), 'Overview of ALDH polymorphism: Relation to cardiovascular effects of alcohol', in: Preedy, V.R. and Watson, R.R. (eds), *Comprehensive Handbook of Alcohol Related Pathology*, Elsevier Academic, London, Vol. 1, pp. 409–424.
4. Zintzaras, E., Stefanidis, I., Santos, M. *et al.* (2006), 'Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease?', *Hepatology* Vol. 43, pp. 352–361.
5. Li, T.-K., Yin, S.-J., Crabb, D.W. *et al.* (2001), 'Genetic and environmental influences on alcohol metabolism in humans', *Alcohol. Clin. Exp. Res.* Vol. 25, pp. 136–144.
6. Edenberg, H.J. and Foroud, T. (2006), 'The genetics of alcoholism: Identifying specific genes through family studies', *Addict. Biol.* Vol. 11, pp. 386–396.
7. Yin, S.-J. and Peng, G.-S. (2007), 'Acetaldehyde, polymorphisms and the cardiovascular system', *Novartis Found. Symp.* Vol. 285, pp. 52–63; discussion, pp. 63–68.
8. Duester, G., Farres, J., Felder, M.R. *et al.* (1999), 'Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family', *Biochem. Pharmacol.* Vol. 58, pp. 389–395.
9. Yin, S.-J., Wang, M.-F., Han, C.-L. *et al.* (1995), 'Substrate binding pocket structure of human aldehyde dehydrogenases: A substrate specificity approach', *Adv. Exp. Med. Biol.* Vol. 372, pp. 9–16.
10. Lee, S.-L., Chau, G.-Y., Yao, C.-T. *et al.* (2006), 'Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: Significance of first-pass metabolism', *Alcohol. Clin. Exp. Res.* Vol. 30, pp. 1132–1142.
11. Niederhut, M.S., Gibbons, B.J., Perez-Miller, S. *et al.* (2001), 'Three-dimensional structures of the three human class I alcohol dehydrogenases', *Protein Sci.* Vol. 10, pp. 697–706.
12. Agarwal, D.P. and Goedde, H.W. (1992), 'Pharmacogenetics of alcohol metabolism and alcoholism', *Pharmacogenetics* Vol. 2, pp. 48–62.
13. Li, H., Mukherjee, N., Soundararajan, U. *et al.* (2007), 'Geographically separate increases in the frequency of the derived ADH1B\*47 His allele in Eastern and Western Asia', *Am. J. Hum. Genet.* Vol. 81, pp. 842–846.
14. Vasiliou, V., Bairoch, A., Tipton, K.F. *et al.* (1999), 'Eukaryotic aldehyde dehydrogenase (ALDH) genes: Human polymorphisms, and recommended nomenclature based on divergent evolution and chromosomal mapping', *Pharmacogenetics* Vol. 9, pp. 421–434.
15. Sophos, N.A., Black, W.J. and Vasiliou, V. (2006), 'An update of the ALDH gene superfamily', in: Weiner, H., Lindahl, R., Plapp, B.V. *et al.* (eds), *Enzymology and Molecular Biology of Carbonyl Metabolism 12*, Purdue University, West Lafayette, IN, pp. 3–7.
16. Deitrich, R.A., Petersen, D. and Vasiliou, V. (2007), 'Removal of acetaldehyde from the body', *Novartis Found. Symp.* Vol. 285, pp. 23–40; discussion pp. 40–51.
17. Larson, H.N., Zhou, J., Chen, Z. *et al.* (2007), 'Structural and functional consequences of coenzyme binding to the inactive Asian variant of mitochondrial aldehyde dehydrogenase: Roles of residues 475 and 487', *J. Biol. Chem.* Vol. 282, pp. 12940–12950.
18. Zhou, J. and Weiner, H. (2000), 'Basis for half-of-the-site reactivity and the dominance of the K487 Oriental subunit over the E487 subunit in heterotetrameric human liver mitochondrial aldehyde dehydrogenase', *Biochemistry* Vol. 39, pp. 12019–12024.
19. Chen, C.-C., Lu, R.-B., Chen, Y.-C. *et al.* (1999), 'Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism', *Am. J. Hum. Genet.* Vol. 65, pp. 795–807.

20. Higuchi, S., Matsushita, S., Imazeki, H. *et al.* (1994), 'Aldehyde dehydrogenase genotypes in Japanese alcoholics', *Lancet* Vol. 343, pp. 741–742.
21. Chen, Y.-C., Lu, R.-B., Peng, G.-S. *et al.* (1999), 'Alcohol metabolism and cardiovascular response in an alcoholic patient homozygous for the *ALDH2\*2* variant gene allele', *Alcohol. Clin. Exp. Res.* Vol. 23, pp. 1853–1860.
22. Kim, D.J., Choi, I.G., Park, B.L. *et al.* (2008), 'Major genetic components underlying alcoholism in Korean population', *Hum. Mol. Genet.* Vol. 17, pp. 854–858.
23. Edenberg, H.J., Xuei, X., Chen, H.-J. *et al.* (2006), 'Association of alcohol dehydrogenase genes with alcohol dependence: A comprehensive analysis', *Hum. Mol. Genet.* Vol. 15, pp. 1539–1549.
24. Wall, T.L., Carr, L.G. and Ehlers, C.L. (2003), 'Protective association of genetic variation in alcohol dehydrogenase with alcohol dependence in native American mission Indians', *Am. J. Psychiatry* Vol. 160, pp. 41–46.
25. Choi, I.-G., Son, H.-G., Yang, B.-H. *et al.* (2005), 'Scanning of genetic effects of alcohol metabolism gene (*ADH1B* and *ADH1C*) polymorphisms on the risk of alcoholism', *Hum. Mutat.* Vol. 26, pp. 224–234.
26. Luo, X., Kranzler, H.R., Zuo, L. *et al.* (2006), 'Diplotype trend regression analysis of the *ADH* gene cluster and the *ALDH2* gene: Multiple significant associations with alcohol dependence', *Am. J. Hum. Genet.* Vol. 78, pp. 973–987.
27. Kuo, P.-H., Kalsi, G., Prescott, C.A. *et al.* (2008), 'Association of *ADH* and *ALDH* genes with alcohol dependence in the Irish affected sib pair study of alcohol dependence (Iaspsad) sample', *Alcohol. Clin. Exp. Res.* Vol. 32, pp. 785–795.
28. Lee, S.-L., Höög, J.-O. and Yin, S.-J. (2004), 'Functionality of allelic variations in human alcohol dehydrogenase gene family: Assessment of a functional window for protection against alcoholism', *Pharmacogenetics* Vol. 14, pp. 725–732.
29. Peng, G.-S., Wang, M.-F., Chen, C.-Y. *et al.* (1999), 'Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians', *Pharmacogenetics* Vol. 9, pp. 463–476.
30. Peng, G.-S., Yin, J.-H., Wang, M.-F. *et al.* (2002), 'Alcohol sensitivity in Taiwanese men with different alcohol and aldehyde dehydrogenase genotypes', *J. Formos. Med. Assoc.* Vol. 101, pp. 769–774.
31. Peng, G.-S., Chen, Y.-C., Tsao, T.-P. *et al.* (2007), 'Pharmacokinetic and pharmacodynamic basis for partial protection against alcoholism in Asians heterozygous for the variant *ALDH2\*2* gene allele', *Pharmacogenet. Genomics* Vol. 17, pp. 845–855.
32. Crabb, D.W., Matsumoto, M., Chang, D. *et al.* (2004), 'Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology', *Proc. Nutr. Soc.* Vol. 63, pp. 49–63.
33. Yin, S.-J., Lee, S.-L., Yao, C.-T. *et al.* (2007), 'Functional roles of alcohol dehydrogenases in human ethanol metabolism', in: Weiner, H., Lindahl, R., Plapp, B.V. *et al.* (eds), *Enzymology and Molecular Biology of Carbonyl Metabolism 13*, Purdue University, West Lafayette, IN, pp. 134–143.
34. Nuutinen, H.U., Salaspuro, M.P., Valle, M. *et al.* (1984), 'Blood acetaldehyde concentration gradient between hepatic and antecubital venous blood in ethanol-intoxicated alcoholics and controls', *Eur. J. Clin. Invest.* Vol. 14, pp. 306–311.
35. Yin, S.-J., Liao, C.-S., Wu, C.-W. *et al.* (1997), 'Human stomach alcohol and aldehyde dehydrogenases: Comparison of expression pattern and activities in alimentary tract', *Gastroenterology* Vol. 112, pp. 766–775.
36. Mizoi, Y., Yamamoto, K., Ueno, Y. *et al.* (1994), 'Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenases in individual variation of alcohol metabolism', *Alcohol Alcohol.* Vol. 29, pp. 707–710.
37. Eriksson, C.J.P. (2007), 'Measurement of acetaldehyde: What levels occur naturally and in response to alcohol?', *Novartis Found. Symp.* Vol. 285, pp. 247–255; discussion pp. 256–260.
38. Neumark, Y.D., Friedlander, Y., Durst, R. *et al.* (2004), 'Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population', *Alcohol. Clin. Exp. Res.* Vol. 28, pp. 10–14.