Published in final edited form as:

Adv Exp Med Biol. 2015 ; 815: 375–387. doi:10.1007/978-3-319-09614-8_22.

Transgenic Mouse Models for Alcohol Metabolism, Toxicity and Cancer

Claire Heit^a, Hongbin Dong^a, Ying Chen^a, Yatrik M. Shah^c, David C. Thompson^b, and Vasilis Vasiliou^a

^aDepartment of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

^bDepartment of Clinical Pharmacy, School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

^cDepartment of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI 48109

Abstract

Alcohol abuse leads to tissue damage including a variety of cancers; however, the molecular mechanisms by which this damage occurs remains to be fully understood. The primary enzymes involved in ethanol metabolism include alcohol dehydrogenase (ADH), cytochrome P450 isoform 2E1, (CYP2E1), catalase (CAT), and aldehyde dehydrogenases (ALDH). Genetic polymorphisms in human genes encoding these enzymes are associated with increased risks of alcohol-related tissue damage, as well as differences in alcohol consumption and dependence. Oxidative stress resulting from ethanol oxidation is one established pathogenic event in alcohol-induced toxicity. Ethanol metabolism generates free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and has been associated with diminished glutathione (GSH) levels as well as changes in other antioxidant mechanisms. In addition, the formation of protein and DNA adducts associated with the accumulation of ethanol-derived aldehydes can adversely affect critical biological functions and thereby promote cellular and tissue pathology. Animal models have proven to be valuable tools for investigating mechanisms underlying pathogenesis caused by alcohol. In this review, we provide a brief discussion on several animal models with genetic defects in alcohol metabolizing enzymes and GSH synthesizing enzymes and their relevance to alcohol research.

I. Introduction

The Centers for Disease Control and Prevention reported that the annual number of alcohol related deaths was 88,000 in the United States from 2006–2010. Alcohol is a causal factor in more than 60 human diseases and places a significant burden on the economy with healthcare costs estimated in 2006 as surpassing 223 billion dollars in the United States alone [1]. A comprehensive understanding of the mechanisms mediating alcohol toxicity is

Corresponding Author: Vasilis Vasiliou, Ph.D. Professor of Molecular Toxicology, Director of Toxicology Graduate Program, Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Denver, 12850 East Montview Boulevard, Aurora, CO 80045, USA. Tel.: +1 303 724 3520; fax: +1 303 724 726.

essential because it facilitates the development of therapies that prevent and/or treat the pathologies associated with alcohol consumption. The cellular and molecular mechanisms leading to alcohol-induced tissue damage are not fully understood. However, emerging evidence indicates that common mechanisms of cell injury, such as stress, inflammation, and alterations in signaling (including apoptosis) pathways, are all involved in the deleterious effects of alcohol.

Animals in which expression of specific proteins are repressed, so-called knockout animals, represent an innovative and powerful research tool for scientific discovery. Genetic manipulation of proteins involved in the metabolism of ethanol or in the cellular defense mechanisms against alcohol-induced oxidative stress have allowed the exploration of their roles in alcohol-related pathologies, such as alcoholic liver disease, pancreatitis, cardiovascular disease, and diabetes mellitus, as well as in various cancers, including oral, colorectal, liver, pancreatic, aerodigestive, breast and colon [2–7]. Currently available animal models will be outlined in the following review. In addition, double and triple knockout strains of these mice are currently being produced in our laboratory.

II. Clinical significance of human polymorphisms of genes involved in ethanol metabolism

Ethanol is metabolized primarily *via* oxidation to acetaldehyde through the enzymatic activity of alcohol dehydrogenases (ADH), catalase (CAT) and cytochrome p450 2E1 (CYP2E1) (Fig. 1). Acetaldehyde is then oxidized to acetate by the aldehyde dehydrogenases (ALDHs). The role of ADH in ethanol metabolism is well established [8–10]. The human genome contains three Class I *ADH* genes (*ADH1A*, *ADH1B*, *ADH1C*); in contrast, rodents have only one *Adh1* gene [11]. Genetic polymorphisms in *ADH1* genes are associated with colon and breast cancers [4, 5, 12]. The role of CYP2E1 in ethanol metabolism, oxidative injury and cancer is also well established [13–17] and genetic polymorphisms are associated with increased cancer risk [18–20]. Catalase appears to play an important role in ethanol metabolism in the brain [14, 15]. Nevertheless, polymorphisms in the catalase gene are associated with diabetes mellitus, hypertension and vitiligo [21, 22]. ALDHs are a family of 19 human proteins that metabolize aldehydes, in which 3 isoforms are responsible for metabolizing acetaldehyde. Mitochondrial ALDH2 is the primary enzyme involved in the metabolism of acetaldehyde ($K_m = 5\mu M$). The ALDH2*2 allele (which appears restricted to an Asian genetic background) causes marked reductions in acetaldehyde metabolism that manifest clinically as flushing syndrome and ethanol avoidance in heterozygous and homozygous individuals. This polymorphism is also associated with alcohol-related cancers [3, 23]. ALDH1B1 has the next lowest K_m for acetaldehyde ($K_m = 55\mu M$), implicating a role in acetaldehyde metabolism secondary to ALDH2. ALDH1B1 has been proposed as a biomarker for colon cancer [24]. Several ALDH1B1 polymorphisms have been found in humans and recent studies have linked these polymorphisms to drinking aversion, elevated systolic blood pressure and frequent hypersensitivity reactions in Caucasians [25, 26]. ALDH1A1 has a role in acetaldehyde metabolism and drinking preference [27, 28] and a deficiency in this enzyme is associated with ethanol hypersensitivity in Caucasian subjects [29]. Polymorphisms in alcohol- and

acetaldehyde- metabolizing enzymes have been closely linked with alcohol related cancers. In a Japanese population, p53 accumulation, esophageal neoplasia and esophageal squamous cell carcinomas were increased in subjects whose genes included the inactive heterozygous allele ALDH2 *1/*2 and the less active ADH1B *1/*1 [30, 31]. These polymorphisms also exhibited more frequent acetaldehyde induced DNA damage [32].

Taken together, the association between human polymorphisms of ethanol metabolizing genes and alcohol-related diseases implicates a significant pathogenic role of ethanol metabolism in alcohol toxicity. Various research groups have developed animal models that harbor genetic ablations of ethanol-metabolizing enzymes. These models can serve as important experimental tools to elucidate the mechanistic roles of specific enzymes or pathways in alcohol-related diseases and therefore have direct relevance to alcohol research.

III. Animal models for alcohol-induced cancer

Low to moderate consumption of alcohol has tissue protective properties [33]. However, heavy alcohol consumption increases the risk of several diseases, including cancer. A comprehensive review of epidemiological data demonstrated a significant increase in cancer risk for several epithelial-derived tumors associated with ethanol consumption [3]. Studies using experimental animals, however, support the notion that ethanol acts as a cocarcinogen or tumor promoter rather than being a carcinogen itself [5]. The mechanisms by which alcohol promotes tumorigenesis remain unclear due primarily to a lack of good animal models that can recapitulate the increased risk of alcohol in carcinogenesis. Animal models analogous to inflammation-promoted cancers, such as the azoxymethane (AOM)/dextran sulfate sodium (DSS) model, are needed [34]. In this model, the carcinogen AOM by itself is administered at a dose that causes no dysplasia; however, when administered in combination with DSS (which induces inflammation), a synergistic increase in the number of tumors is observed. The creation of a similar model for alcohol will rely on a better understanding of the interactions between the genetic mutations (or carcinogen) with the duration, route and concentration of ethanol for the epithelial tumor that is to be modeled. Moreover, diets that better mimic heavy alcohol consumption in humans are required. The interaction of tumor-promoting dietary components, such as alcohol, high-fat and iron, may lead to more robust and precise models. Lastly, experimental evidence indicates that the metabolism of ethanol leading to the generation of acetaldehyde and free radicals is intimately involved in alcohol-associated carcinogenesis [3]. Therefore, a more comprehensive understanding of the enzymes required for alcohol metabolism in cancer are needed and the genetic animal models discussed in this review could represent unique opportunities to identify their roles in alcohol-induced cancers.

IV. Glutathione in alcoholic tissue injury

In the development of alcohol-induced tissue injury, it is apparent that numerous pathways in target organs are modulated by ethanol [35, 36]. Oxidative stress appears to play a central role in many of these pathways [37]. Ethanol metabolism, CYP2E1 induction, compromised antioxidant defense, mitochondrial injury, inflammation, hypoxia, and iron overload can all contribute to the alcohol-induced oxidative environment. Accumulation of the reactive

molecules (including reactive oxygen species and electrophilic products, such as acetaldehyde and lipid peroxidation products) can be harmful to a biological system due to their propensity to inactivate enzymes and cause DNA damage, loss of protein functions and cell death [38].

Glutathione (GSH) plays an important role as an antioxidant by serving as a cofactor for antioxidant enzymes, such as glutathione peroxidase and glutathione S-transferases, or by directly scavenging free radicals [39]. It is the most abundant non-protein thiol, attaining a concentration in the high millimolar range in the liver [40]. Because of its abundance, GSH plays a key role in maintaining cellular redox homeostasis and, therefore, enzymes that help generate GSH are critical in protecting cells against oxidative stress. GSH is a tripeptide composed of glutamate, cysteine, and glycine. It is synthesized in most types of cells by two successive enzymatic reactions. The first reaction couples glutamate and cysteine and is catalyzed by glutamate-cysteine ligase (GCL), resulting in the formation of γ -glutamylcysteine (γ -GC) [41] (Fig. 1). The second reaction, catalyzed by GSH synthetase, couples γ -GC with glycine. The formation of γ -GC by GCL is considered rate-limiting in GSH biosynthesis, and GCL has been the principal target of drugs designed to inhibit GSH biosynthesis [41] and to generate mice with GSH deficiency [42].

In higher eukaryotes, GCL in its most catalytically efficient form, is a heterodimer composed of a catalytic (GCLC) and a modifier (GCLM) subunit, each of which is encoded by separate genes on different chromosomes. GCLC possesses all of the catalytic activity of γ -GC formation; GCLM optimizes the kinetic properties of the holoenzyme, thereby regulating tissue GSH levels [43]. GSH is exclusively synthesized in the cytoplasm [39] and further distributed into mitochondria, endoplasmic reticulum and nuclei, where it plays a pivotal role in the normal functioning of these subcellular organelles [44]. During detoxication of free radicals, GSH is oxidized to glutathione disulfide (GSSG). Both GSH and GSSG can be transported outside the cell where it is broken down in sequence by γ -glutamyl transferases and dipeptidase, producing free cysteine and glycine for intracellular reutilization [45]. Depletion of hepatic GSH, particularly mitochondrial GSH, occurs as a result of excessive GSH consumption by free radicals and acetaldehyde generated during alcohol metabolism [46]. Given the above considerations, animal models exhibiting a GSH deficiency will serve as important tools to study GSH-regulated redox biology in ethanol metabolism and ethanol-induced tissue damage. As such, they are of direct relevance to alcohol research.

V. Mouse models with genetic deficiencies in ethanol-metabolizing enzymes

ADH1 global knockout

The *Adh1*^{-/-} mouse line has been generated by Duester [47] (Table 1). It should be noted that human *ADH1* gene family consists of three genes, viz. *ADH1A*, *ADH1B* and *ADH1C*, whereas the mouse genome has a single *Adh1* gene [11]. *Adh1*^{-/-} mice have limited capacity to oxidize ethanol and retinol. Pharmacokinetic studies show a reduction in blood ethanol clearance in these animals [9]. Following parenteral administration of ethanol, these

mice displayed an increased sleep time and embryonic resorption was increased 3-fold [9]. While ADH1 is thought to be responsible for the majority of ethanol metabolism in the liver, new pharmacokinetic evidence suggests a role for other ADH isoforms as well [48]. Therefore, this model may be useful in determining the pathophysiological importance of compensatory ADH isoforms as well as elucidating the kinetics of these enzymes for ethanol.

Catalase global knockout

The *Cat*^{-/-} mouse strain was developed and characterized by Ho and colleagues [49] (Table 1). These mice do not express catalase and develop normally, i.e., exhibit no gross abnormalities. However, brain mitochondria of these animals show deficiencies in mitochondrial respiration. To date, this knockout strain has not been subjected to ethanol toxicity studies. Given that earlier studies have shown a significant role of catalase in modulating ethanol sensitivity in the brain [14, 15], the *Cat*^{-/-} mice would be anticipated to be a valuable animal model for examining ethanol drinking preference as well as alcohol toxicities.

CYP2E1 global knockout

CYP2E1 is an ethanol-inducible enzyme with a role in hepatic ethanol oxidation. By genetically ablating exon 2 of *Cyp2e1* gene, Gonzalez and colleagues developed *Cyp2e1*^{-/-} mice [50] (Table 1). These mice do not express CYP2E1 enzyme and develop normally [50]. They also show lower sensitivity to the deleterious hepatic effects of acetaminophen [50]. As one of the primary xenobiotic/endobiotic metabolizing p450s, CYP2E1 is a contributor to a variety of cellular toxicities induced by endogenous or exogenous pathogens. Using the *Cyp2e1*^{-/-} mouse model, CYP2E1 has been shown to play a pivotal role in mediating hepatotoxicity making this an interesting model for alcohol-related liver toxicity [51, 52]. *Cyp2e1*^{-/-} and *Cyp2e1* knock-in mice have been used to examine the potentiation of ethanol-induced hypoxia. *Cyp2e1*^{-/-} mice exhibited the lowest levels of hypoxia and HIF1- α [53]. Similarly, ethanol-induced fatty liver and oxidant stress are blunted in these mice [54]; this study confirmed the important role of CYP2E1 in ethanol-induced liver toxicities. *Cyp2e1*^{-/-} mice also display longer ethanol-induced sleep time than do wild-type mice [15], confirming the relevance of the *Cyp2e1*^{-/-} mouse line for the study of the CYP2E1 enzyme in ethanol toxicities and alcohol-induced drinking preference.

VI. Mouse models with genetic deficiencies in acetaldehyde-metabolizing enzymes

ALDH2 global knockout

The *Aldh2*^{-/-} strain was originally developed and characterized by Isse and colleagues [55, 56] (Table 1). *Aldh2*^{-/-} mice do not express ALDH2 protein and have no detectable capacity to oxidize acetaldehyde, propionaldehyde or methoxyacetaldehyde in liver mitochondrial fractions. Following oral administration of ethanol, *Aldh2*^{-/-} mice exhibit higher ethanol and acetaldehyde levels and lower acetate levels in the blood, brain and liver than *Aldh2*^{+/+} mice [57, 58]. Further, they are more susceptible to ethanol-induced body

weight loss [59], but show no change in mortality [60]. *Aldh2*^{-/-} mice are more sensitive to the toxic effects of inhaled acetaldehyde [61] and exhibit more frequent mutations in the T cell receptor site than their corresponding wild-type [62]. A single oral dose of ethanol in *Aldh2*^{-/-} downregulates the alcohol-metabolizing CYP2E1 mRNA [63], which suggests that there is compensation due to an abundance of acetaldehyde. This treatment has also been shown to decrease hepatic malondialdehyde and increase hepatic glutathione, both markers of oxidative stress, in *Aldh2*^{-/-} mice [64]. Acetaldehyde adducts are also increased in *Aldh2*^{-/-} mice. These mice have been used to determine ethanol- and acetaldehyde-induced cholinergic changes in the hippocampus. The null mice exhibit decreases in choline acetyltransferase mRNA and protein; however, neurotrophins (nerve growth factor or brain-derived neurotrophic factor) remain unaffected [65], indicating that aldehydes have a selective effect in the brain. *Aldh2*^{-/-} mice also exhibit alcohol avoidance in a test of preference and difference in liver or brain acetaldehyde levels [55]. ALDH2 also appears to influence bone growth and cardiac function, as demonstrated by reductions in trabecular bone formation and cardiomyocyte function in *Aldh2*^{-/-} mice treated with alcohol [66, 67]. Stomach DNA adducts are dramatically increased after chronic ethanol feeding of *Aldh2*^{-/-} mice [68, 69] and acute ethanol treatment increases hepatic oxidative DNA adducts in null mice [70, 71]. The *Aldh2*^{-/-} strain represents a valuable strain that can be used to identify functions of ALDH2 in ethanol metabolism and toxicity.

ALDH2 conditional knockout

A “knockout-first” conditional allele for *Aldh2* has been developed by Skarnes and colleagues [72]. These mice have been crossed with *FLP* mice to generate *Aldh2* floxed conditional knockout (*Aldh2*^{ff}) mice, which can be further crossed with specific *CRE* mouse lines to generate cell-specific *Aldh2* knockout mice. As expected, *Aldh2*^{ff} mice develop normally and exhibit no observed phenotype (unpublished observation). To date, no ethanol studies have been conducted in these mice. This strain can be used to study tissue-specific contributions of ALDH2 in ethanol metabolism and toxicity.

ALDH1B1 global knockout

The *Aldh1b1*^{-/-} strain was recently generated by Vasiliou and coworkers (Singh S, Vasiliou V *et al*, manuscript in preparation) (Table 1). The *Aldh1b1*^{-/-} mice develop normally and show no overt phenotype. In agreement with the catalytic properties of ALDH1B1 (i.e., the second lowest *K_m* for acetaldehyde oxidation [73]), these mice exhibit higher blood concentrations of acetaldehyde following acute ethanol administration [manuscript in preparation]. The *Aldh1b1*^{-/-} strain represents the first animal model for the study of ALDH1B1 enzyme in ethanol-induced tissue injury.

ALDH1A1 global knockout

The *Aldh1a1*^{-/-} mouse line has been generated by Fan *et al* [74] (Table 1). These mice are fertile and exhibit no overt phenotype, except that aged *Aldh1a1*^{-/-} mice display ~2.4-fold higher cataract incidence than wild-type mice [75]. While ALDH1A1 primarily metabolizes retinaldehyde, it also plays a role in acetaldehyde metabolism. Genetic variants of *ALDH1A1* (that result in low enzyme activity) have been associated with increased alcohol

sensitivity in Caucasians [29]. Therefore, the *Aldh1a1*^{-/-} mouse line represents a useful animal model for investigation of the ALDH1A1 enzyme in ethanol toxicities.

VII. Mouse models with GSH deficiency

GCLC conditional (*Gclc*^{fl/fl}) knockout

The global gene knockout of *Gclc* results in embryonic lethality, indicating an essential role of GSH in mouse development [76]. The *Gclc*^{fl/fl} strain was developed and originally characterized by Chen and colleagues [77]. The *in vivo* role of hepatic GSH has been investigated using the hepatocyte-specific *Gclc* knockout (*Gclc*^{h/h}) mice created by intercrossing *Gclc*^{fl/fl} and *Alb-Cre* mice [77]. *Gclc*^{h/h} mice experience almost complete loss of hepatic GSH (~5% of normal) and die from acute liver failure when mitochondrial failure occurs [77]. Chronic administration of N-acetylcysteine, a treatment that promotes only a mild increase in liver GSH levels (to 8% of normal) but partially preserves mitochondrial function, allows *Gclc*^{h/h} mice to survive to adulthood, albeit with the serious liver pathologies fibrosis and cirrhosis [78]. These studies demonstrate an essential role of GSH in normal functioning of the liver. The *Gclc*^{fl/fl} mice represent a unique model that can be used to elucidate cell-specific functions of GSH in ethanol metabolism and toxicity.

GCLM global knockout

The *Gclm*^{-/-} strain was developed and originally characterized by Yang and coworkers [79]. *Gclm*^{-/-} mice are viable and fertile, despite having only 9–16% of the normal GSH levels in liver, lung, pancreas, erythrocytes, and plasma [79]. Except when challenged with oxidant stress [80, 81], *Gclm*^{-/-} mice exhibit no overt phenotype, making them a useful model for studying chronic GSH depletion. Interestingly, these mice show accelerated clearance of ethanol and acetaldehyde and are protected from alcohol-induced steatosis (Chen Y, Vasiliou V et al, manuscript in preparation). Thus, *Gclm*^{-/-} mice represent a model wherein significant GSH depletion in the liver is associated with beneficial metabolic and stress responses to ethanol.

VIII. Concluding remarks

Alcohol use is widespread and related to numerous diseases, including oral, colorectal, liver, pancreas, aerodigestive, breast and colon cancers. Ethanol metabolism and resultant oxidative stress are primary pathogenic events mediating alcohol-induced organ damage and neurobehavioral changes, the molecular details of which are not yet fully understood. The knockout mouse models for enzymes metabolizing ethanol (ADH1, CAT and CYP2E1), acetaldehyde (ALDH2, ALDH1A1 and ALDH1B1) and enzymes involved in GSH synthesis (GCLC and GCLM), which we have discussed briefly in this review, represent unique and highly relevant animal models for alcohol research. Utilization of these models will deliver valuable information about the fundamental mechanisms underlying ethanol toxicity. Such knowledge should accelerate the development of more effective, targeted therapies to both prevent and treat health issues associated with excessive alcohol consumption.

Acknowledgements

This work was supported in part by the National Institutes of Health Grants No. R24AA022057, No. R01EY14390, No. T32AA007464, CA148828, and DK095201.

References

1. Bouchery EE, et al. Economic costs of excessive alcohol consumption in the U.S., 2006. *Am J Prev Med.* 2011; 41(5):516–524. [PubMed: 22011424]
2. McKillop IH, Schrum LW. Role of alcohol in liver carcinogenesis. *Seminars in Liver Disease.* 2009; 29(2):222–232. [PubMed: 19387921]
3. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nature Reviews Cancer.* 2007; 7(8):599–612.
4. Visapaa JP, et al. Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. *Gut.* 2004; 53(6):871–876. [PubMed: 15138216]
5. Poschl G, Seitz HK. Alcohol and cancer. *Alcohol Alcohol.* 2004; 39(3):155–165. [PubMed: 15082451]
6. Boffetta P, Hashibe M. Alcohol and cancer. *Lancet Oncology.* 2006; 7(2):149–156.
7. Reidy J, McHugh E, Stassen LF. A review of the relationship between alcohol and oral cancer. *Surgeon.* 2011; 9(5):278–283. [PubMed: 21843823]
8. Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Res Health.* 2007; 30(1):5–13. [PubMed: 17718394]
9. Deltour L, Foglio MH, Duester G. Metabolic deficiencies in alcohol dehydrogenase Adh1, Adh3, and Adh4 null mutant mice. Overlapping roles of Adh1 and Adh4 in ethanol clearance and metabolism of retinol to retinoic acid. *Journal of Biological Chemistry.* 1999; 274(24):16796–16801. [PubMed: 10358022]
10. Scott DM, Taylor RE. Health-related effects of genetic variations of alcohol-metabolizing enzymes in African Americans. *Alcohol Res Health.* 2007; 30(1):18–21. [PubMed: 17718396]
11. Duester G, et al. Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. *Biochem Pharmacol.* 1999; 58(3):389–395. [PubMed: 10424757]
12. Lilla C, et al. Alcohol dehydrogenase 1B (ADH1B) genotype, alcohol consumption and breast cancer risk by age 50 years in a German case-control study. *British Journal of Cancer.* 2005; 92(11):2039–2041. [PubMed: 15886702]
13. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radical Biology and Medicine.* 2008; 44(5):723–738. [PubMed: 18078827]
14. Zimatkin SM, et al. Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol Clin Exp Res.* 2006; 30(9):1500–1505. [PubMed: 16930212]
15. Vasiliou V, et al. CYP2E1 and catalase influence ethanol sensitivity in the central nervous system. *Pharmacogenet Genomics.* 2006; 16(1):51–58. [PubMed: 16344722]
16. Millonig G, et al. Ethanol-mediated carcinogenesis in the human esophagus implicates CYP2E1 induction and the generation of carcinogenic DNA-lesions. *International Journal of Cancer.* 2011; 128(3):533–540.
17. Wang Y, et al. Ethanol-induced cytochrome P4502E1 causes carcinogenic etheno-DNA lesions in alcoholic liver disease. *Hepatology.* 2009; 50(2):453–461. [PubMed: 19489076]
18. Trafalis DT, et al. CYP2E1 and risk of chemically mediated cancers. *Expert Opin Drug Metab Toxicol.* 2010; 6(3):307–319. [PubMed: 20073996]
19. Druesne-Pecollo N, et al. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. *Lancet Oncology.* 2009; 10(2):173–180.
20. Morita M, et al. Genetic polymorphisms of CYP2E1 and risk of colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Epidemiology, Biomarkers and Prevention.* 2009; 18(1):235–241.
21. Goth L, Nagy T. Acatalasemia and diabetes mellitus. *Archives of Biochemistry and Biophysics.* 2012

22. Goth L, Rass P, Pay A. Catalase enzyme mutations and their association with diseases. *Molecular Diagnosis*. 2004; 8(3):141–149. [PubMed: 15771551]
23. Seitz HK, et al. Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. *Gastroenterology*. 1990; 98(2):406–413. [PubMed: 2295396]
24. Chen Y, et al. Aldehyde dehydrogenase 1B1 (ALDH1B1) is a potential biomarker for human colon cancer. *Biochem Biophys Res Commun*. 2011; 405(2):173–179. [PubMed: 21216231]
25. Husemoen LL, et al. The association of ADH and ALDH gene variants with alcohol drinking habits and cardiovascular disease risk factors. *Alcohol Clin Exp Res*. 2008; 32(11):1984–1991. [PubMed: 18782342]
26. Linneberg A, et al. Genetic determinants of both ethanol and acetaldehyde metabolism influence alcohol hypersensitivity and drinking behaviour among Scandinavians. *Clin Exp Allergy*. 2010; 40(1):123–130. [PubMed: 20205700]
27. Little RG 2nd, Petersen DR. Subcellular distribution and kinetic parameters of HS mouse liver aldehyde dehydrogenase. *Comparative Biochemistry and Physiology. C: Comparative Pharmacology*. 1983; 74(2):271–279.
28. Bond SL, Wigle MR, Singh SM. Acetaldehyde dehydrogenase (Ahd-2)-associated DNA polymorphisms in mouse strains with variable ethanol preferences. *Alcoholism: Clinical and Experimental Research*. 1991; 15(2):304–307.
29. Eriksson CJ. The role of acetaldehyde in the actions of alcohol (update 2000). *Alcoholism: Clinical and Experimental Research*. 2001; 25(5):15S–32S. Suppl ISBRA.
30. Yokoyama A, et al. Development of squamous neoplasia in esophageal iodine-unstained lesions and the alcohol and aldehyde dehydrogenase genotypes of Japanese alcoholic men. *Int J Cancer*. 2012; 130(12):2949–2960. [PubMed: 21796615]
31. Yokoyama A, et al. p53 protein accumulation, iodine-unstained lesions, and alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 genotypes in Japanese alcoholic men with esophageal dysplasia. *Cancer Lett*. 2011; 308(1):112–117. [PubMed: 21601984]
32. Yukawa Y, et al. Combination of ADH1B*2/ALDH2*2 polymorphisms alters acetaldehyde-derived DNA damage in the blood of Japanese alcoholics. *Cancer Sci*. 2012; 103(9):1651–1655. [PubMed: 22703580]
33. Arranz S, et al. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. *Nutrients*. 2012; 4(7):759–781. [PubMed: 22852062]
34. Tanaka T. Colorectal carcinogenesis: Review of human and experimental animal studies. *J Carcinog*. 2009; 8:5. [PubMed: 19332896]
35. Altamirano J, Bataller R. Alcoholic liver disease: pathogenesis and new targets for therapy. *Nat Rev Gastroenterol Hepatol*. 2011; 8(9):491–501. [PubMed: 21826088]
36. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology*. 2011; 141(5):1572–1585. [PubMed: 21920463]
37. Albano E. Oxidative mechanisms in the pathogenesis of alcoholic liver disease. *Mol Aspects Med*. 2008; 29(1–2):9–16. [PubMed: 18045675]
38. Bergendi L, et al. Chemistry, physiology and pathology of free radicals. *Life Sciences*. 1999; 65(18–19):1865–1874. [PubMed: 10576429]
39. Meister A. Metabolism and function of glutathione: an overview. *Biochemical Society Transactions*. 1982; 10(2):78–79. [PubMed: 6121735]
40. Kretzschmar M. Regulation of hepatic glutathione metabolism and its role in hepatotoxicity. *Experimental and Toxicologic Pathology*. 1996; 48(5):439–446. [PubMed: 8765689]
41. Meister A. Glutathione metabolism and its selective modification. *Journal of Biological Chemistry*. 1988; 263(33):17205–17208. [PubMed: 3053703]
42. Dalton TP, et al. Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radical Biology and Medicine*. 2004; 37(10):1511–1526. [PubMed: 15477003]
43. Chen Y, et al. Glutamate cysteine ligase catalysis: dependence on ATP and modifier subunit for regulation of tissue glutathione levels. *The Journal of biological chemistry*. 2005; 280(40):33766–33774. [PubMed: 16081425]

44. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB Journal*. 1999; 13(10):1169–1183. [PubMed: 10385608]
45. Meister A. Glutathione metabolism. *Methods in Enzymology*. 1995; 251:3–7. [PubMed: 7651209]
46. Wu D, Cederbaum AI. Oxidative stress and alcoholic liver disease. *Seminars in Liver Disease*. 2009; 29(2):141–154. [PubMed: 19387914]
47. Molotkov A, et al. Distinct retinoid metabolic functions for alcohol dehydrogenase genes *Adh1* and *Adh4* in protection against vitamin A toxicity or deficiency revealed in double null mutant mice. *Journal of Biological Chemistry*. 2002; 277(16):13804–13811. [PubMed: 11836246]
48. Haseba T, Ohno Y. A new view of alcohol metabolism and alcoholism--role of the high-Km Class III alcohol dehydrogenase (ADH3). *Int J Environ Res Public Health*. 2010; 7(3):1076–1092. [PubMed: 20617019]
49. Ho YS, et al. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *Journal of Biological Chemistry*. 2004; 279(31):32804–32812. [PubMed: 15178682]
50. Lee SS, et al. Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J Biol Chem*. 1996; 271(20):12063–12067. [PubMed: 8662637]
51. Valentine JL, et al. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. *Toxicol Appl Pharmacol*. 1996; 141(1):205–213. [PubMed: 8917693]
52. Wong FW, Chan WY, Lee SS. Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. *Toxicol Appl Pharmacol*. 1998; 153(1):109–118. [PubMed: 9875305]
53. Wang X, et al. Cytochrome P450 2E1 potentiates ethanol induction of hypoxia and HIF-1 α in vivo. *Free Radic Biol Med*. 2013; 63:175–186. [PubMed: 23669278]
54. Lu Y, et al. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. *Hepatology*. 2008; 47(5):1483–1494. [PubMed: 18393316]
55. Isse T, et al. Diminished alcohol preference in transgenic mice lacking aldehyde dehydrogenase activity. *Pharmacogenetics*. 2002; 12(8):621–626. [PubMed: 12439222]
56. Yu HS, et al. Characteristics of aldehyde dehydrogenase 2 (*Aldh2*) knockout mice. *Toxicol Mech Methods*. 2009; 19(9):535–540. [PubMed: 19874182]
57. Kiyoshi A, et al. Ethanol metabolism in ALDH2 knockout mice--blood acetate levels. *Leg Med (Tokyo)*. 2009; 11(Suppl 1):S413–S415. [PubMed: 19356968]
58. Isse T, et al. Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity shows high acetaldehyde level in blood, brain, and liver after ethanol gavages. *Alcohol Clin Exp Res*. 2005; 29(11):1959–1964. [PubMed: 16340452]
59. Oyama T, et al. A pilot study on subacute ethanol treatment of ALDH2 KO mice. *J Toxicol Sci*. 2007; 32(4):421–428. [PubMed: 17965556]
60. Isse T, et al. Aldehyde dehydrogenase 2 activity affects symptoms produced by an intraperitoneal acetaldehyde injection, but not acetaldehyde lethality. *Journal of Toxicological Sciences*. 2005; 30(4):315–328. [PubMed: 16404140]
61. Oyama T, et al. Susceptibility to inhalation toxicity of acetaldehyde in *Aldh2* knockout mice. *Front Biosci*. 2007; 12:1927–1934. [PubMed: 17127431]
62. Kunugita N, et al. Increased frequencies of micronucleated reticulocytes and T-cell receptor mutation in *Aldh2* knockout mice exposed to acetaldehyde. *J Toxicol Sci*. 2008; 33(1):31–36. [PubMed: 18303182]
63. Matsumoto A, et al. Single-dose ethanol administration downregulates expression of cytochrome p450 2E1 mRNA in aldehyde dehydrogenase 2 knockout mice. *Alcohol*. 2007; 41(8):587–589. [PubMed: 17980998]
64. Matsumoto A, et al. Lack of aldehyde dehydrogenase ameliorates oxidative stress induced by single-dose ethanol administration in mouse liver. *Alcohol*. 2007; 41(1):57–59. [PubMed: 17452299]
65. Jamal M, et al. Ethanol- and acetaldehyde-induced cholinergic imbalance in the hippocampus of *Aldh2*-knockout mice does not affect nerve growth factor or brain-derived neurotrophic factor. *Brain Res*. 2013; 1539:41–47. [PubMed: 24096209]

66. Ma H, et al. Aldehyde dehydrogenase 2 knockout accentuates ethanol-induced cardiac depression: role of protein phosphatases. *J Mol Cell Cardiol.* 2010; 49(2):322–329. [PubMed: 20362583]
67. Shimizu Y, et al. Reduced bone formation in alcohol-induced osteopenia is associated with elevated p21 expression in bone marrow cells in aldehyde dehydrogenase 2-disrupted mice. *Bone.* 2011; 48(5):1075–1086. [PubMed: 21256255]
68. Nagayoshi H, et al. Increased formation of gastric N(2)-ethylidene-2'-deoxyguanosine DNA adducts in aldehyde dehydrogenase-2 knockout mice treated with ethanol. *Mutat Res.* 2009; 673(1):74–77. [PubMed: 19101651]
69. Matsumoto A, et al. Effects of 5-week ethanol feeding on the liver of aldehyde dehydrogenase 2 knockout mice. *Pharmacogenet Genomics.* 2008; 18(10):847–852. [PubMed: 18704003]
70. Kim YD, et al. Ethanol-induced oxidative DNA damage and CYP2E1 expression in liver tissue of Aldh2 knockout mice. *J Occup Health.* 2007; 49(5):363–369. [PubMed: 17951967]
71. Matsuda T, et al. Increased formation of hepatic N2-ethylidene-2'-deoxyguanosine DNA adducts in aldehyde dehydrogenase 2-knockout mice treated with ethanol. *Carcinogenesis.* 2007; 28(11):2363–2366. [PubMed: 17361010]
72. Skarnes WC, et al. A conditional knockout resource for the genome-wide study of mouse gene function. *Nature.* 2011; 474(7351):337–342. [PubMed: 21677750]
73. Stagos D, et al. Aldehyde dehydrogenase 1B1: molecular cloning and characterization of a novel mitochondrial acetaldehyde-metabolizing enzyme. *Drug Metab Dispos.* 2010; 38(10):1679–1687. [PubMed: 20616185]
74. Fan X, et al. Targeted disruption of Aldh1a1 (Raldh1) provides evidence for a complex mechanism of retinoic acid synthesis in the developing retina. *Mol Cell Biol.* 2003; 23(13):4637–4648. [PubMed: 12808103]
75. Lassen N, et al. Multiple and additive functions of ALDH3A1 and ALDH1A1: cataract phenotype and ocular oxidative damage in Aldh3a1(–/–)/Aldh1a1(–/–) knock-out mice. *J Biol Chem.* 2007; 282(35):25668–25676. [PubMed: 17567582]
76. Shi ZZ, et al. Glutathione synthesis is essential for mouse development but not for cell growth in culture. *Proc Natl Acad Sci U S A.* 2000; 97(10):5101–5106. [PubMed: 10805773]
77. Chen Y, et al. Hepatocyte-specific Gclc deletion leads to rapid onset of steatosis with mitochondrial injury and liver failure. *Hepatology.* 2007; 45(5):1118–1128. [PubMed: 17464988]
78. Chen Y, et al. Oral N-acetylcysteine rescues lethality of hepatocyte-specific Gclc-knockout mice, providing a model for hepatic cirrhosis. *J Hepatol.* 2010; 53(6):1085–1094. [PubMed: 20810184]
79. Yang Y, et al. Initial characterization of the glutamate-cysteine ligase modifier subunit Gclm(–/–) knockout mouse. Novel model system for a severely compromised oxidative stress response. *Journal of Biological Chemistry.* 2002; 277(51):49446–49452. [PubMed: 12384496]
80. McConnachie LA, et al. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. *Toxicological Sciences.* 2007; 99(2):628–636. [PubMed: 17584759]
81. Chen Y, et al. Glutathione-deficient mice are susceptible to TCDD-Induced hepatocellular toxicity but resistant to steatosis. *Chem Res Toxicol.* 2012; 25(1):94–100. [PubMed: 22082335]

Fig. 1. Major enzymatic pathways involved in ethanol and glutathione metabolism

Ethanol (EtOH) is subject to metabolism by catalase (CAT), cytochrome P450 isoform 2E1 (CYP2E1) and alcohol dehydrogenase (ADH). Acetaldehyde is metabolized by aldehyde dehydrogenase (ALDH) isoforms 1A1, 1B1 and 2. In the glutathione (GSH) pathway, glutamate cysteine ligase (GCL), which includes two subunits the catalytic subunit (GCLC) and the modifier subunit (GCLM), catalyzes the synthesis of γ -glutamylcysteine (γ -GC). γ -GC is then coupled to glycine by glutathione synthetase (GSS) to form GSH. During oxidative processes, reactive oxygen species (ROS) form which can cause lipid peroxidation. ROS can be reduced by GSH, in the process forming GSSG, the oxidized form.

Table 1**Transgenic mouse models**

A variety of transgenic strains are available for research. For each strain, the genetic background and phenotypes are provided.

<i>Strain</i>	Genetic background	Phenotype	Reference
<i>Adh1</i> ^{-/-}	C57BL6	<ul style="list-style-type: none"> No gross abnormality Reduction in blood ethanol clearance 	Deltour et al 1999
<i>Cat</i> ^{-/-}	C57BL6	<ul style="list-style-type: none"> No gross abnormality Deficiency in brain mitochondrial respiration Has not been used in ethanol toxicity studies 	Ho et al 2004
<i>Cyp2e1</i> ^{-/-}	129/Sv	<ul style="list-style-type: none"> No gross abnormality Decreased sensitivity to acetaminophen hepatotoxicity Resistance to ethanol-induced fatty liver and oxidant stress 	Lee et al 1996, Lu et al 2008
<i>Cat</i> ^{-/-} / <i>Cyp2e1</i> ^{-/-} double knockout	C57BL6/129 mixed	<ul style="list-style-type: none"> No gross abnormality Has not been used in ethanol toxicity studies 	Unpublished
<i>Aldh2</i> ^{-/-}	C57BL6	<ul style="list-style-type: none"> No gross abnormality High susceptibility to ethanol toxicities by oral administration High sensitivity to inhalation toxicities of acetaldehyde 	Isse et al 2002, Oyama et al 2007
<i>Aldh1a1</i> ^{-/-}	C57BL6	<ul style="list-style-type: none"> Viable and fertile Decreased susceptibility to diet-induced obesity and insulin resistance Cataract development at age of 6-month Has not been used in ethanol toxicity studies 	Fan et al 2003, Ziouzenkova et al 2007
<i>Aldh1b1</i> ^{-/-}	C57BL6	<ul style="list-style-type: none"> No gross abnormality Reduction in blood acetaldehyde clearance 	Unpublished