4. Mechanistic and Other Relevant Data Relevant

4.1 Absorption, first-pass metabolism, distribution and excretion

4.1.1 *Humans*

(a) Ethanol

(i) *Absorption*

After oral ingestion, alcohol is slowly absorbed by the stomach, but is rapidly absorbed by simple diffusion once it passes into the small intestine. The oral pharmacokinetics of ethanol is subject to large interindividual variation in blood alcohol concentrations, even when the dose of ethanol is adjusted for gender and given to subjects who have fasted or have received a standardized meal before the dose (O'Connor *et al.*, 1998). Total volumes of body water and liver per unit of lean body mass should be taken into consideration as factors that influence the results of metabolic studies of ethanol. Since women have more fat and less body water per unit of lean body mass, they have higher blood alcohol concentrations than men after a dose of ethanol based on total body weight. Men and women have nearly identical peak blood alcohol concentrations after the same dose of alcohol per unit of total body water (Goist & Sutker, 1985). Some studies still found higher alcohol elimination rates in women, despite adjustment of the dose for total body water (Thomasson *et al.*, 1995). Women have a proportionately larger volume of liver per unit of lean body mass than men. When alcohol elimination rates were obtained by the intravenous steady-state infusion method, no gender difference was found in the rates per unit of liver volume (Kwo *et al.*, 1998).

The variation in blood alcohol concentrations after meals is even more complicated, because of the changes in first-pass metabolism with gender and age, and the ability of some common drugs (aspirin, cimetidine) to reduce first-pass metabolism (Roine *et al.*, 1990; Caballeria *et al.*, 1989a). This, plus the well known inaccuracy of self-reported alcoholic beverage consumption, complicates attempts to correlate different levels of reported alcoholic beverage drinking with the overall risk for cancer,

or with specific cancers (i.e. to generate estimated dose–response curves or predict safe levels of drinking).

(ii) *First-pass metabolism*

First-pass metabolism is represented by the difference between the quantity of a drug (ethanol) consumed orally and the amount that reaches the systemic circulation. Conceptually, first-pass metabolism is due to metabolism of ethanol in the gastrointestinal mucosa or liver during its passage through these tissues. It reduces the amount of ethanol that reaches target organs. The gut contains cytochrome P450s (CYPs) and alcohol dehydrogenases (ADHs). Ethanol is absorbed slowly from the stomach and is therefore subject to oxidation, while the ethanol that leaves the stomach is very rapidly absorbed from the upper small intestine, leaving little time for metabolism by that tissue. After absorption, ethanol travels to the liver, where a certain percentage is metabolized before passing into the *vena cava* (Julkunen *et al.*, 1985; Caballeria *et al.*, 1987). The relative proportion of first-pass metabolism is greatest with low doses of ethanol (0.3 g/kg bw, equivalent to approximately 20 g ethanol or two social drinks) when gastric emptying is slowed down (typically by the presence of food). Larger doses of ethanol or rapid gastric emptying reduce the difference between the areas under the curve (AUCs), which may then be too small to measure accurately. The phenomenon of first-pass metabolism is well established, but there remains debate about the relative contribution of the stomach and the liver (Lim *et al.*, 1993). The gastric mucosa expresses ADH isozymes (ADH1C, ADH5 and ADH7; see Section 4.2.1) that can oxidize ethanol. Gastric ADH activity was decreased in certain populations, e.g. in women (Frezza *et al.*, 1990; Seitz *et al.*, 1993), in individuals with atrophic gastritis and in alcoholics (DiPadova *et al.*, 1987; Pedrosa *et al.*, 1996) and in individuals who used medication (Caballeria *et al.*, 1989a; Roine *et al.*, 1990; Caballería, 1992); under these circumstances, the magnitude of first-pass metabolism was reduced. ADH7, a major gastric ADH isozyme, had low activity in endoscopic mucosal biopsies of the stomach in about 46% of Asians. Those who have lower ADH7 enzyme activity had lower rates of first-pass metabolism (Dohmen *et al.*, 1996), which suggests that ADH7 participates in the gastric oxidation of ethanol. In addition, higher rates of gastric emptying yielded higher peak blood alcohol concentrations and AUCs, and lower rates of first-pass metabolism (Holt, 1981). Combinations of type of alcoholic beverage, volume and concentration with the prandial state influence the rate of gastric emptying of alcohol and the resulting blood alcohol concentrations and AUCs (Roine *et al.*, 1991, 1993; Roine, 2000). The fact that first-pass metabolism is reduced when gastric emptying is rapid suggests that contact of alcohol with the stomach favours the absorption of alcohol across the mucosa, where it would be subject to oxidation. Oral intake of alcohol caused significantly higher blood alcohol concentrations and AUC in the fasted as compared with the fed state (DiPadova *et al.*, 1987). All of these reports are consistent with a role for the stomach mucosa in first-pass metabolism of ethanol.

Levitt and Levitt (2000) have pointed out that calculating first-pass metabolism from the AUCs is valid when the elimination of the drug under consideration is first-order, and that ethanol is cleared by zero-order kinetics for most of the elimination curve. They argued, with the use of a two-compartment model, that first-pass metabolism is only observed at very low doses of alcohol that does not cause inebriation (Levitt & Levitt, 1998). They also found that only a small fraction of ethanol absorbed from the stomach is metabolized in humans, and that most first-pass metabolism is hepatic (Levitt *et al.*, 1997a). The assertion that gastric ADH (Yin *et al.*, 1997) or first-pass metabolism (Ammon *et al.*, 1996) is reduced in women has been contested. Some investigators found no correlation between gastric ADH activity and first-pass metabolism (Brown *et al.*, 1995). Further, the total ADH activity in the stomach, calculated from the mass of the mucosa and its ADH activity, does not account for the differences between the AUCs of oral and intravenous intake of alcohol caused by the degree of ethanol metabolism (Yin *et al.*, 1997). Additionally, while humans and rats have similar first-pass metabolism ratios, their gastric ADHs have markedly different kinetic properties. The Michaelis constant (K_m) for ethanol of the human enzyme is 40 mM, while that of the rat enzyme is 5M (~125 times greater). These arguments suggest that first-pass metabolism of ethanol also occurs in the liver. Hepatic first-pass metabolism depends on the rate of ethanol absorption because portal alcohol concentration depends on the rate of absorption. Low rates of absorption and low portal venous ethanol concentrations would permit ethanol to be extensively oxidized by the low-K_m hepatic ADH isozymes. At higher rates of absorption and higher portal ethanol concentrations, these enzymes are saturated soon after drinking begins.

Ammon *et al.* (1996) compared the metabolic fates of ethanol given intravenously and deuterated ethanol given orally or into the duodenum. Since individuals served as their own controls, this reduced the intra-subject variability. First-pass metabolism accounted for about 8–9% of the oral dose, and the gastric contribution was estimated to be about 6% of the oral dose.

In summary, first-pass metabolism of orally ingested ethanol usually contributes a small fraction (up to 10% when a small dose of ethanol is consumed) of its total body elimination. When gastric emptying is rapid or the ethanol dose consumed is high, first-pass metabolism is quantitatively less important and, similarly, gender differences are probably not a major factor (Ammon *et al.*, 1996). The importance of demonstrating gastric first-pass metabolism, even though it may be small in magnitude, lies in the potential for local metabolism of ethanol in the digestive tract and in the likelihood that ADHs with a higher K_m are active at the high concentrations of ethanol achieved in the stomach (Caballeria *et al.*, 1989b; Roine 2000). An extensive discussion of the different metabolic pathways of ethanol is given in section 4.2.

(iii) *Distribution and excretion*

Ethanol is distributed throughout the total body water. After the distribution phase, the concentration of ethanol in the saliva (Gubała & Zuba, 2002) and in the colon is the same as that in the blood (Halsted *et al.*, 1973).

It has been estimated that over 90% of the elimination of ethanol occurs through oxidation in the liver. The remaining elimination is a combination extrahepatic oxidation and losses of small amounts of ethanol in the breath (0.7%), sweat (0.1%) and urine (0.3–4%) (Holford, 1987; Ammon *et al.*, 1996). The rate of removal of ethanol from the blood in the pseudo-linear segment of the elimination curve varies by two- to three-fold between individuals (Kopun & Propping, 1977; Martin *et al.*, 1985). This large interindividual variation was recently confirmed by use of the alcohol clamp technique (O'Connor *et al.*, 1998). The reasons for this variation are incompletely understood, but probably include variation in the size of the liver, in the activity of enzymes that catalyse alcohol oxidation or in the steady-state concentrations of substrates and products. Kwo *et al.* (1998) determined that the metabolic rate of ethanol correlated well with liver volume measured by quantitative tomography scanning, and that the higher rate of elimination of ethanol reported in women (when expressed on the basis of body weight) was accounted for by the fact that women have similarly sized livers to men, and thus a larger liver:body weight ratio.

Ramchandani *et al.* (2001) reported that elimination of ethanol (measured by means of alcohol clamping) could be accelerated by about 50% by ingestion of a meal, and that the composition of the meal was not important in this effect. [The Working Group noted the surprising result of this study, and considered that replication is needed.] This effect may be the result of changes in liver blood flow or possibly in the intrahepatic redox state. The polymorphic ADH enzymes (see below) have also been considered to contribute to this variability in the metabolic rates of alcohol.

(b) Acetaldehyde

Acetaldehyde is metabolized by aldehyde dehydrogenases (ALDHs), which are widely expressed in the mitochrondria and cytosol of most tissues (reviewed in Crabb, 1995), especially the mitochondrial form with a low K_m, so that almost all of the acetaldehyde produced by hepatic metabolism of ethanol is converted into acetate in the liver (reviewed in Gemma *et al.*, 2006). Chronic ethanol consumption is reported to reduce ALDH activity in the livers of alcoholics and to elevate blood acetaldehyde concentrations (reviewed in Nuutinen *et al.*, 1983, 1984); interpretation of the latter finding is complicated by the fact that red blood cells also present ALDH activity. A useful five-compartment physiologically-based pharmacokinetic model has recently been developed for quantitative analysis of acetaldehyde clearance (Umulis *et al.*, 2005).

4.1.2 *Experimental systems*

(a) Ethanol

Lim *et al.* (1993) examined the effect of infusion of ethanol into the pylorus-ligated stomach, duodenum or portal vein of rats and found that first-pass metabolism was only noted when ethanol was administered into the stomach. Experimentally, the systemic AUC of ethanol concentration is very sensitive to the rate of portal venous administration of ethanol (Smith *et al.*, 1992; Levitt *et al.*, 1994), which also accounts for the lack of first-pass metabolism with high doses of ethanol or rapid gastric emptying and therefore rapid delivery of ethanol to the liver. Only small differences in ethanol metabolites were found across the stomach in rats. Levitt *et al.* (1997b) found negligible oxidation of ethanol in the gastric mucosa as it was absorbed from the pylorus-ligated stomach in rats. This controversy was reviewed by Crabb (1997).

(b) Acetaldehyde

In rats, chronic treatment with 30% ethanol in the drinking-water or with an acute dose of 5 g/kg bw caused increases in specific activities of low-K_m and high-K_m ALDH in hepatic mitochondria (Aoki & Itoh, 1989). Feeding rats with a liquid diet containing alcohol resulted in a significant reduction in low K_m ALDH in the rectum but no change in the stomach, small intestine or colon; high-K_m ALDH was not altered in any tissue (Pronko *et al.*, 2002). Induced CYP2E1 may also act on acetaldehyde: liver microsomes from starved or acetone-treated rats exhibited an eightfold increase in acetaldehyde metabolism, with a K_m of 30 μM and a maximum velocity (V_{max}) of 6.1 nmol/mg/min, and this activity was inhibited by anti-CYP2E1 antibody (Terelius *et al.*, 1991). However, CYP2E1 activity towards acetaldehyde was much lower than that towards ethanol and was markedly inhibited by ethanol, which suggests that, under normal conditions, CYP2E1 probably does not play a major role in acetaldehyde metabolism (Wu *et al.*, 1998).

4.2 Metabolism

4.2.1 *Humans*

(a) Ethanol

In this section, tissue distribution of ADHs and other enzymes that oxidize ethanol and generate or oxidize acetaldehyde are reviewed, in order to assess which tissues are probably subject to the eventual carcinogenic effects of ethanol and acetaldehyde.

(i) *Alcohol dehydrogenase (ADH) pathway*

General description

The enzymes responsible for the major part of ethanol oxidation are the ADHs. All are dimeric enzymes with a subunit molecular weight of about 40 kDa; subunits

are identified by Greek letters. They are grouped into classes based upon enzymatic properties and the degree of sequence similarities. Enzyme subunits that belong to the same class can heterodimerize. Class I contains α, β and γ isozymes that are encoded by *ADH1A, ADH1B* and *ADH1C* genes. These enzymes have a low K_m for ethanol and are highly sensitive to inhibition by pyrazole derivatives. They are very abundant in the liver, and play a major role in the metabolism of alcohol. Class II ADH (π ADH, encoded by *ADH4*) is also abundant in the liver, has a higher K_m for ethanol and is less sensitive to inhibition by pyrazole than class I enzymes (Ehrig *et al.*, 1990). Class III ADH (χ ADH, encoded by *ADH5*) is present in nearly all tissues, is virtually inactive with ethanol but can metabolize longer-chain alcohols, α-hydroxy-fatty acids and formaldehyde (as a GSH-dependent formaldehyde dehydrogenase). A recent study suggested that class III ADH may be more active towards ethanol in a hydrophobic environment, and argued that liver cytosol may be such an environment (Haseba *et al.*, 2006). The class IV enzyme, σ-ADH, was purified from the stomach and oesophagus (Parés *et al.*, 1994). σ-ADH, the product of the *ADH7* gene, has the highest V_{max} of the known ADHs and is very active towards retinol, an activity that is shared by class I ADHs. This may be relevant to its expression in numerous epithelia that are dependent on retinol for their integrity. Class V ADH, encoded by the *ADH6* gene, is expressed in the liver and in the stomach, but the enzyme itself has not been purified (Yasunami *et al.*, 1991). The enzyme expressed *in vitro* has a high K_m for ethanol (about 30 mM) and moderate sensitivity to pyrazole inhibition (Chen & Yoshida, 1991).

Human ADHs

Variation in the *ADH* genes is unique to humans. The isozymes in class I are polymorphic; two alleles exist for *ADH1C* and three for *ADH1B* (Burnell & Bosron, 1989). The kinetic properties and geographical distribution of these allelic enzymes are shown in Table 4.1. The isozymes encoded by the three *ADH1B* alleles, each differing from the others at a single amino acid residue, vary markedly in K_m for ethanol and in V_{max}. Subunit β1 is most common in Caucasians and has a relatively low V_{max} and a very low K_m for ethanol. Subunit β2 is found commonly in Asians and was originally designated 'atypical' ADH. This gene is common among Ashkenazi Jews in Israel and the USA (Neumark *et al.*, 1998; Shea *et al.*, 2001; Hasin *et al.*, 2002). It has a substantially higher V_{max} and somewhat higher K_m than β1. The β3 isozyme was first detected in liver extracts from African-Americans on the basis of its lower pH optimum than that of the other ADH isozymes. It has also been found in Southwest American Indians and in groups of African origin in the Caribbean. It has a high K_m for ethanol and high V_{max}. Smaller differences in enzymatic properties are observed between the products of the *ADH1C* alleles. The V_{max} of the γ1 isozyme is about twice as high as that of the γ2 isozyme, while the K_ms (K_m at half saturation) for ethanol are similar. The γ1 ADH isozyme is found at high frequency in Asians and African-Americans; Caucasians have about an equal frequency of γ1 and γ2 *ADH* alleles (Burnell & Bosron, 1989; Bosron & Li, 1986). A variant of *ADH1C* (with a threonine at position 351) was detected in Native American populations, but not in Europeans or Africans; the kinetic effect

of this variant is unknown (Osier *et al*., 2002). Variants of ADH4 (corresponding to ADH2 in the new nomenclature; see Duester *et al*., 1999) were recently described in a Swedish population (Strömberg *et al*., 2002). A substitution of valine for isoleucine at position 308 was detected; the valine variant was less thermostable *in vitro*, but its kinetic properties were similar.

The widely varying V_{max} and K_m of the ADH1B and ADH1C isozymes suggest the possibility that individuals with different combinations of isozymes have different rates of elimination of ethanol. The presence of more active ADH isozymes was predicted to increase the rates of ethanol metabolism. This has been difficult to demonstrate, in part because a given isozyme constitutes only a fraction of the total capacity of the liver to oxidize ethanol and because the elimination rates of ethanol are rather variable even among individuals of the same *ADH* genotypes, or even twins (Kopun & Propping, 1977; Martin *et al*., 1985). To date, different *ADH1B* genotypes have been related to only a small portion of the intra-individual differences in ethanol elimination rates (Mizoi *et al*., 1994; Thomasson *et al*., 1995; Neumark *et al*., 2004). The *ADH1B*3* polymorphism has been shown to be associated with an approximate 15% increase in the rate of ethanol metabolism. Both *ADH1B*2* and *ADH1B*3* are protective against alcoholism (Edenberg *et al*., 2006). The *ADH1C* polymorphism did not affect the elimination of ethanol (Couzigou *et al*., 1991). It has not been possible to demonstrate increased blood levels of acetaldehyde in individuals with the higher-activity ADH enzymes except in individuals with inactive ADH2 (see below).

The ADH isozymes that have a high K_m for ethanol, e.g. β3, π and σ, are predicted to be more active when blood ethanol concentrations are high or in tissues of the upper gastrointestinal tract that are directly exposed to alcoholic beverages. Increased clearance of ethanol was seen in baboons with high blood ethanol concentrations (Pikkarainen & Lieber, 1980). This has not been tested directly in humans to date because of ethical concerns, but studies of intoxicated individuals indicated a more rapid elimination rate of ethanol when blood ethanol levels were higher (Brennan *et al*., 1995; Jones & Andersson, 1996).

An additional *ADH* genetic variant is a *Pvu* II restriction fragment length polymorphism in an intron of the *ADH1B* gene. It is not known whether the variant alters expression of the gene or is linked to another susceptibility locus; the *B* allele was found at a higher frequency in alcoholics and in patients with alcoholic cirrhosis (Sherman *et al*., 1993b). Single nucleotide polymorphisms (SNPs) that are presumed to influence expression of the *ADH4* gene (*ADH2* in the new nomenclature; Duester *et al*., 1999) have been linked to the risk for alcoholism (Edenberg *et al*., 2006); one polymorphism in the promoter affects gene expression (Edenberg *et al*., 1999). Similarly, sequence variants in the promoter of *ADH1C* may affect its expression (Chen *et al*., 2005a).

Tissue distribution of ADH

In humans, the liver expresses the highest levels of class I, II and III, which is consistent with the role of the liver in the elimination of ethanol. However, the enzymes are expressed in several other tissues, and may play a role in the toxicity or carcinogenicity

Table 4.1 Biochemical properties of human alcohol dehydrogenase (ADH)[a] and acetaldehyde dehydrogenase (ALDH)

Gene locus	Allele	Protein subunit	K_m	$V_{max}=(k_{cat})$	Ethnic/national distribution	References
			K_m ethanol (mM)			
ADH1A	*ADH1A*	α	4.2	27	Europe, Africa	Burnell & Bosron (1989); Ehrig *et al.* (1990)
ADH1B	*ADH1B*1*	β1	0.05	9	Europe, Africa	Bosron & Li (1986); Thomasson *et al.* (1995)
	*ADH1B*2*	β2	0.9	400	Asia	
	*ADH1B*3*	β3	34	300	Africa, Native American	
ADH1C	*ADH1C*1*	γ1	1.0	87	All	
	*ADH1C*2*	γ2	0.63	35	Europe	
	*ADH1C*3*		NR	NR	Native American	Osier *et al.* (2002)
ADH4	*ADH4*1*	π	34	40	All	
	*ADH4*2*		10.6	10.5	Sweden	Strömberg *et al.* (2002)
ADH5		χ	1000		All	
ADH6		NPT	30	NR	All	
ADH7		σ, μ	20	1510	All	

Table 4.1 (continued)

Gene locus	Allele	Protein subunit	K_m	$V_{max}=(k_{cat})$	Ethnic/national distribution	References
			K_m acetaldehyde (μM)			
ALDH1A1			30		All	
ALDH2	*ALDH2*1*		1		All	
	*ALDH2*2*				Asia	Crabb *et al.* (1989)
	*ALDH2*3*				Taiwan, China	Novoradovsky *et al.* (1995a)
ALDH1B1	*ALDH1B1*1*		NR			
(ALDH5)	*ALDH1B1*2*		NR			Sherman *et al.* (1993a)
ALDH9A1	*ALDH9A1*1*		30		All	Kurys *et al.* (1989)
	*ALDH9A1*2*					Lin *et al.* (1996)

k_{cat}, constant of turnover rate of enzyme-substance complex; K_m, Michaelis constant; NR, not reported; NPT, not purified from tissue; Vmax, maximum velocity

[a] For nomenclature of ADHs, see Duester *et al.* (1999); ADH1A, ADH1B and ADH1C are the new nomenclature of ADH1, ADH2 and ADH3 (old nomenclature), respectively. ADH4 is the old nomenclature of ADH2, ADH5 is the old nomenclature of ADH6 and ADH7 is the old nomenclature of ADH4 (see Duester *et al.*, 1999). The kinetic constants are noted for the homodimers of the ADH subunits listed (heterodimers behave as if the active sites were independent). The K_m values are in mM (ethanol) for ADH and μM (acetaldehyde) for ALDH, and the V_{max} values for ADHs are given in terms of turnover numbers (min^{-1}) for comparison. The column labelled ethnic/national distribution indicates which populations have high allele frequencies for these variants. The alleles are not limited to these populations.

of ethanol in those tissues. This has been studied in enzyme assays that use a variety of substrates to distinguish partially the various isozymes, and by use of northern blotting to assess mRNA levels. However, in the two studies, total class I *ADH* mRNA was analysed (i.e. by probing the blots with an *ADH1B* or *ADH1C* cDNA), which thus does not allow an understanding of locus-specific expression (see Table 4.2). Class I *ADH* is expressed in several tissues, in particular in the gastrointestinal tract (Yin *et al.*, 1993; Seitz *et al.*, 1996; Yin *et al.*, 1997), salivary glands (Väkeväinen *et al.*, 2001) and mammary gland (Triano *et al.*, 2003). Breast tissue expresses mRNA that corresponds to class I ADH and contains immunoreactive class I ADH by immunohistochemistry (localized to the mammary epithelial cells) and western blotting. These assays did not differentiate between ADH1A, ADH1B and ADH1C. Activity assays revealed the presence of ADH that is maximally active with 10 mM ethanol and can be inhibited with 4-methylpyrazole (Triano *et al.*, 2003). These characteristics are consistent with the presence of the *ADH1B* gene product, β-ADH (Triano *et al.*, 2003) or the *ADH1C* gene product, γ-ADH. Conversely, Gene Expression Omnibus (GEO) (microarray) profiles (www.ncbi.nih.gov) indicate the presence of *ADH1B* transcripts in breast tissue. Individuals homozygous for *ADH1C*1* had higher levels of acetaldehyde in the saliva after an alcohol challenge (Visapää *et al.*, 2004). Class IV is expressed at highest levels in the gums, tongue, oesophagus and stomach (Yin *et al.*, 1993; Dong *et al.*, 1996). Gastric mucosa contains several ADHs (γ-, σ- and μ-ADH) (Yin *et al.*, 1997), but σ-ADH was absent in the stomach biopsies from about 80% of Asians. Those who lacked this enzyme had a lower first-pass metabolism of ethanol (Dohmen *et al.* 1996), which suggests that σ-ADH is important in the gastric oxidation of ethanol. The mechanism for this deficiency has not been discovered, despite sequencing of exons in various ethnic groups. The human colon expresses ADH1C in the mucosa and, very weakly, ADH1B in the smooth muscle (Yin *et al.*, 1994). The relative expression of various *ADH* mRNAs can be estimated from the frequency of expressed sequence tags detected in cDNA libraries, which permits assessment of the probable level of expression of ADH enzymes in less accessible tissues. Figure 4.1 shows a compilation of data on the expression of *ADH1C, ADH4, ADH6* and *ADH7* transcripts in human tissues. These data may be subject to error due to the presence of repetitive elements. While not of human origin, there is a large mass of microorganisms in the gastrointestinal tract that may contribute to ethanol oxidation and the local formation of acetaldehyde. Microorganisms express numerous forms of ADH, which can contribute to the formation of acetaldehyde in the lower gastrointestinal tract or wherever microbial overgrowth occurs.

Variation of expression of ADH

In humans, the amount of ADH in the liver is not induced by chronic alcohol drinking before the development of liver disease (Panés *et al.*, 1989); however, with fasting, protein malnutrition and liver disease, ADH activity and the rate of ethanol elimination are decreased. Orchiectomy increased rates of ethanol elimination in humans (Mezey *et al.*, 1988). Little is known about the expression of extrahepatic ADH, with

Table 4.2 Distribution of alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) mRNAs in human tissues

Enzyme	mRNA	No mRNA detected	References
Class I (ADH1A, ADH1B, ADH1C)	Liver, lung, stomach, ileum, colon, uterus, kidney, spleen, skin, testis, ovary, cervix, heart, skeletal muscle, pancreas, prostate, adrenal cortex and medulla, thyroid, blood vessels (intima and media: mainly ADH1B detected as isozyme protein and activity)	Brain, placenta, peripheral blood leukocytes	Engeland & Maret (1993); Estonius *et al*. (1996); Allali-Hassani *et al*. (1997)
Class II (ADH4)	Liver, small intestine, pancreas, stomach, testis, kidney		Engeland & Maret (1993); Estonius *et al*. (1996)
Class III (ADH5)	All tissues examined		
Class IV (ADH7)	Stomach (other epithelial tissues not examined); small intestine, fetal liver highest of all		Yokoyama *et al*. (1995); Estonius *et al*. (1996)
ADH5	Liver, small intestine, fetal kidney; fetal liver highest of all		Estonius *et al*. (1996)
ALDH1A1	Liver, lung, kidney, skeletal muscle, pancreas; lower in testis, prostate, ovary, lung, small intestine		Stewart *et al*. (1996a)
ALDH2	Fetal heart, brain, liver, lung, kidney; adult liver, kidney, skeletal and cardiac muscle, lung; lower in pancreas		Stewart *et al*. (1996a)
ALDH1B1 (ALDH5)	Fetal heart, brain, liver, lung, kidney; adult liver, skeletal muscle, kidney; lower in brain, placenta, prostate, gut, lung, pancreas, ovary, testis		Stewart *et al*. (1996a)
ALDH9A1	Liver, skeletal muscle, kidney; low levels in heart, pancreas, placenta, lung, brain		Lin *et al*. (1996)

the exception of gastric ADH, which is reduced in women under 50 years of age who are heavy drinkers according to some investigators (Seitz *et al*., 1993) but not others (Yin *et al*., 1997).

(ii) *Microsomal oxidation pathway*

General description

Ethanol can be metabolized by microsomal ethanol-oxidizing systems, predominantly via CYP2E1. Other cytochrome-associated enzymes, CYP1A2 and CYP3A4, contribute to a lesser extent (Lieber, 2004a). Hamitouche *et al.* (2006) demonstrated that a wide variety of recombinant human CYP isoforms expressed in baculovirus-infected insect cells, with the exception of CYP2A6 and 2C18, can oxidize ethanol to

Figure 4.1. Tissue distribution of alcohol dehydrogenase (*ADH*), cytochrome P450 2E1 (*CYP2E1*) and catalase (*CAT*) transcripts reflected by the abundance of expressed sequence tags

Tissue	*ADH1C*	*ADH4*	*ADH6*	*ADH7*	*CYP2E1*	*CAT*
Adipose tissue	4251	0	0	0	0	144
Adrenal gland	611	0	0	0	0	32
Blood	0	17	0	0	53	367
Bone	13	0	0	0	13	55
Bone marrow	0	0	0	0	0	634
Brain	27	0	1	0	19	47
Cervix	62	0	20	0	0	41
Colon	153	0	14	0	0	84
Connective tissue	74	0	0	0	0	65
Eye	9	0	0	19	0	67
Heart	602	0	55	0	0	100
Kidney	56	0	84	0	0	79
Larynx	32	0	0	32	0	98
Liver	1930	729	252	0	843	319
Lung	169	0	0	40	28	69
Lymph node	10	0	0	0	0	146
Mammary gland	450	29	23	0	29	58
Muscle	122	0	8	17	8	69
Nerve tissue	550	0	0	0	39	118

Figure 4.1. (contd)

Tissue	*ADH1C*	*ADH4*	*ADH6*	*ADH7*	*CYP2E1*	*CAT*
Oesophagus	472	0	52	996	0	0
Ovary	0	0	9	0	28	0
Pancreas	36	4	4	0	0	95
Pharynx	0	0	0	0	0	0
Placenta	16	0	0	0	0	121
Prostate	32	0	0	0	6	51
Salivary gland	0	0	48	0	0	146
Skin	21	0	0	0	0	85
Small intestine	1558	22	90	0	0	22
Spleen	416	0	0	0	0	37
Stomach	254	0	48	9	0	19
Testis	28	0	11	0	8	48
Thymus	135	0	0	0	13	0
Thyroid	0	0	0	0	18	163
Tongue	30	0	15	90	0	30
Trachea	1444	0	0	288	0	20
Urinary bladder	132	0	0	33	0	99
Uterus	217	0	8	0	4	62
Vascular	118	0	0	0	0	157

The number given for each tissue is the abundance of the expressed sequence tag in terms of transcripts/million.
This Figure is compiled from information publicly available at the National Center for Biotechnology Information (NCBI) (see http://www.ncbi.nlm.nih.gov/unigene)

acetaldehyde, with K_ms of approximately 10 mM. CYP2E1 is associated with nicotinamide-adenine dinucleotide phosphate (NADPH)-CYP reductase in the endoplasmic reticulum, and reduces molecular oxygen to water as ethanol is oxidized to acetaldehyde. Its K_m for ethanol is about 10 mM; thus CYP2E1 may assume a greater role in ethanol metabolism at high blood alcohol levels. CYP2E1 is unusually 'leaky' and generates reactive oxygen species including hydroxyl radical, superoxide anion, hydrogen peroxide and hydroxyethyl radical. Thus, CYP2E1 is a major source of oxidative stress (Caro & Cederbaum, 2004).

Microsomal ethanol-oxidizing systems were originally thought to be implicated in the proliferation of the endoplasmic reticulum proliferation in liver biopsies from alcoholics. This was subsequently shown to be due to increased amounts of the enzyme now designated CYP2E1. CYP2E1 can be induced by chronic alcohol drinking, especially in the perivenular zone, and it may contribute to the increased rates of ethanol elimination in heavy drinkers. CYP2E1 is induced during fasting, by diabetes and by a diet high in fat, which may relate to its ability to oxidize the ketone, acetone (Lieber, 2004b). Liver biopsies of recently drinking alcoholics showed a substantial increase in *CYP2E1* mRNA indicating that pre- and post-translational mechanisms are responsible for the induction of this enzyme (Takahashi *et al.*, 1993).

Tissue distribution

CYP2E1 is expressed at high levels in the liver, as well as numerous other tissues, as demonstrated by western blotting, analysis of mRNA, or expressed sequence- tag analyses (Figure 4.1). The organs include kidney, lung, oesophagus, biliary epithelium, pancreas, uterus, leukocytes, brain, colon and nasal mucosa (Ingelman-Sundberg *et al.*, 1994; Crabb, 1995; McKinnon & McManus, 1996; Nishimura *et al.*, 2003). Western blots and activity assays have confirmed expression of CYP2E1 in the oesophagus, pancreas and lung, among other organs. In the brain, CYP2E1 was reported to be expressed in neurons and was induced by administration of ethanol (Tindberg & Ingelman-Sundberg, 1996). CYP2E1 has also been detected in breast tissue (El Rayes *et al.*, 2003)

Genetic variants

An *Rsa* I (−1019C >T) polymorphism (the *Rsa*I^+ allele is also named the *c1* allele) is located in the 5′-flanking region of the *CYP2E1* gene (Hayashi *et al.*, 1991) in a region that interacts with hepatocyte nuclear factor 1 (HNF-1). The *Rsa*I^- allele (*c2*) was more active in in-vitro transcription assays (Watanabe *et al.*, 1994), although a corresponding increase in CYP2E1 activity *in vivo* has not been confirmed unequivocally, based on the clearance of chlorzoxazone. The frequency of this polymorphism depends on continental origin: the *c2* variant is found in 5–10% of Caucasians and in 35–38% of East Asians (Garte *et al.*, 2001). A meta-analysis suggested a possible increased risk for gastric cancer in Asians homozygous for the *c2* allele (Boccia *et al.*, 2007). Another polymorphism, detectable with the DraI restriction enzyme, is located in intron 6 (Uematsu *et al.*, 1991). The distribution of the variant genotype (lacking the *Dra*I site) also depends on continental origin: 40–50% of East Asians carry this genotype, while

only 8–12% of Caucasians lack the *Dra*I site (Garte *et al*., 2001). A recently described polymorphism is the −71G >T polymorphism in the promoter region of the *CYP2E1* gene, which has been associated with enhanced transcriptional activity of promoter constructs in HepG2 cells (Qiu *et al*., 2004). Heterozygosity for this allele occurs in about 10% of Caucasians (Yang *et al*., 2001). The effects of the various genotypes on the pharmacokinetics of ethanol or the risk for alcoholic complications have been inconsistent.

A 96-base-pair insertion polymorphism is known to occur in the regulatory region of the *CYP2E1* gene. The insertion allele is relatively common in Asians (15%) but less so in Caucasians (2%) (Fritsche *et al*., 2000). The polymorphism was shown to increase the inducibility of CYP2E1 activity, as judged from chloroxazone metabolism, in patients who were obese or who had recently consumed alcoholic beverages (McCarver *et al*., 1998). Other polymorphisms have been catalogued by Agarwal (2001).

Since CYP2E1 has a high K_m for ethanol, it generates more acetaldehyde when ethanol concentrations are elevated. There is no evidence that acetaldehyde is a product inhibitor of CYP2E1; in fact, CYP2E1 can oxidize acetaldehyde to acetate, although probably not in the presence of ethanol.

(iii) *Ethanol oxidation by catalase*

Peroxisomal catalase is a tetrameric, haeme-containing enzyme. In addition to converting hydrogen peroxide to water and oxygen, it can oxidize ethanol to acetaldehyde in a hydrogen peroxide-dependent fashion. This pathway is not thought to be a major elimination pathway under most physiological conditions, but it may be important in certain tissues. Acatalasemic mice had longer sleep times than their normal counterparts (Vasiliou *et al*., 2006), which suggests a role of catalase in the effects of ethanol on the brain. It has been suggested that, by inhibiting fatty acid oxidation in the liver, ethanol shunts fatty acids to the peroxisomal pathway, which leads to the formation of hydrogen peroxide, which in turn increases the ability of catalase to oxidize ethanol. This would be particularly important if it occurred in extrahepatic tissues, since plasma fatty acid levels are increased under some circumstances by alcoholic beverage consumption.

There are only few studies on the role of catalase in the oxidation of ethanol. Catalase is expressed in nearly all tissues, as estimated from data on the abundance of expressed sequence tags (Figure 4.1). Catalase is also expressed by microorganisms in the colon and contributes to the formation of acetaldehyde from ethanol in the lower gastrointestinal tract (Tillonen *et al*., 1998). Absence of active catalase (acatalasaemia) is encountered in Asian populations. Several single nucleotide polymorphisms in the 5′ untranslated region and introns have been reported (Jiang *et al*., 2001), but there are no known effects of these variants on the expression or activity of the enzyme, nor on responses to ethanol.

(iv) *Non-oxidative ethanol metabolism*

Ethanol can be non-oxidatively metabolized to form fatty acid ethyl esters (FAEEs) (Laposata & Lange, 1986), which appear in human serum shortly after consumption of ethanol (Doyle *et al.*, 1994). These esters form during the hydrolysis of fatty acid esters (e.g. triglycerides) in the presence of ethanol; they are toxic to cells (Laposata *et al.*, 2002). Fatty acid ethyl ester synthase (FAEES) activity has been attributed to several distinct enzymes: an anionic form of GST (GST-pi-1) was reported by Bora *et al.* (1991) to be the same as FAEES III from human heart muscle. The purified enzyme has a K_m for ethanol of 300 mM, indicating that, *in vivo*, its activity increases in proportion to cellular ethanol concentration (Bora *et al.*, 1996), and it also exhibits carboxylesterase activity. However, the identity of FAEES as a GST was challenged by Board *et al.* (1993). Additional enzymes with FAEES activity include lipoprotein lipase, carboxylesterase ES10 in the liver and cholesterol esterase in the pancreas (Kaphalia *et al.*, 1997). These enzymes are found in several tissues that are affected by ethanol yet do not have high levels of ethanol-oxidizing enzymes (heart, brain, pancreas). In addition, it has been demonstrated that ethanol can be transferred to fatty acyl-coenzyme A (CoA) by an enzyme called acyl-CoA:ethanol *O*-acyltransferase (AEAT) (Diczfalusy *et al.*, 2001). AEAT activity is high in the human duodenum, pancreas and liver. This distribution of AEAT may explain the appearance of FAEEs in lipoproteins: FAEEs may be formed in the duodenum and intestine during absorption of fat in the presence of ethanol. These enzymes all appear to have a high K_m for ethanol, and thus are more active at high concentrations of ethanol (e.g., in the gut and after heavy drinking).

(v) *Other pathways of ethanol oxidation*

Several minor pathways of acetaldehyde formation have been suggested. Nitric oxide synthases 1 and 2 were reported to generate the 1-hydroxyethyl radical from ethanol in the presence of NADPH and arginine, which is to be expected given the presence of a CYP motif within the structure of the enzymes. The 1-hydroxyethyl radical can break down to form acetaldehyde (Porasuphatana *et al.*, 2006). Castro *et al.* (2001a,b) reported that cytosolic xanthine oxidoreductase can oxidize ethanol to acetaldehyde. CYP reductase (in the absence of specific forms of CYP known to be involved in ethanol metabolism, such as CYP2E1) was reported to oxidize ethanol to the 1-hydroxyethyl radical and acetaldehyde, possibly via the semiquinone form of flavine adenine dinucleotide (Díaz Gómez *et al.*, 2000). Other investigators reported the formation of acetaldehyde from ethanol in tissue extracts for which the responsible enzymes have not been identified or only to a limited extent, in studies with different cofactors and inhibitors (Castro *et al.*, 2002, 2003, 2006). It is possible that other oxidant species (hydroxyl radical) that are formed non-enzymatically may be able to oxidize ethanol to acetaldehyde. In addition, acetaldehyde can be formed during the degradation of threonine, putatively by threonine aldolase (Chaves *et al.*, 2002; Crabb & Liangpunsakul, 2007).

(b) Acetaldehyde

(i) *Acetaldehyde oxidation by ALDHs*

General description

Acetaldehyde is metabolized predominantly by nicotinamide-adenine dinucleotide $(NAD)^+$-dependent ALDHs. These enzymes have broad substrate specificity for aliphatic and aromatic aldehydes, which are irreversibly oxidized to their corresponding carboxylic acids (Vasiliou *et al.*, 2004). The ALDHs are expressed in a wide range of tissues, and their nomenclature has recently been revised. The original designations assigned numbers based on electrophoretic mobility, and different laboratories used different systems. Based on kinetic properties and sequence similarities, the ALDHs have been classified into three groups: class I (ALDH1) is present in the cytosol and has a low K_m for aldehydes; class II (ALDH2) is located in the mitochondria, has a low K_m and is the isozyme responsible for the majority of the further oxidation of acetaldehyde that is formed as a result of ethanol oxidation; and class III (ALDH3 or ALDH4) is present in the cytosol and in microsomes of tumours (stomach and cornea) and has a high K_m (Vasiliou *et al.*, 2000, 2004). In addition to these three groups, the human genes that code for ALDHs have been classified into 18 major families; updated information on classification and chromosome location can be found at: http://www.aldh.org/. In this system, *ALDH1* is designated *ALDH1A1* and *ALDH2* retains the same name. *ALDH3* is renamed *ALDH3A1* and *ALDH4* is designated *ALDH4A1*.

The most important enzymes for ethanol metabolism are cytosolic ALDH1A1 and mitochondrial ALDH2. Both are tetrameric enzymes composed of ~55-kDa subunits. ALDH1A1 has a very low K_m for NAD^+ and a low K_m for acetaldehyde (about 50 μM), and is very sensitive to disulfiram (Antabuse) *in vitro*. ALDH1A1 is involved in ethanol detoxification, metabolism of neurotransmitters and synthesis of retinoic acid (Vasiliou *et al.*, 2004). ALDH2 has a K_m for acetaldehyde less than 5 μM, and is less sensitive to disulfiram *in vitro*. These enzymes have high inhibition constants for reduced NAD (NADH), and thus remain active despite the high $NADH/NAD^+$ ratio established in cytosol and mitochondria during ethanol metabolism.

Numerous other ALDH enzymes have been studied. ALDHE3, which is encoded by the *ALDH9A1* gene (Lin *et al.*, 1996), has properties similar to ALDH1A: it is expressed in the cytosol and has a K_m for aliphatic aldehydes of about 30–50 μM (Kurys *et al.*, 1989). It has a low K_m for aminoaldehydes such as 4-aminobutyraldehyde, and hence may play a role in the metabolism of compounds derived from polyamines such as spermine, as well as trimethylaminobutyraldehyde in the synthesis of carnitine. It also oxidizes betaine aldehyde efficiently (Chern & Pietruszko, 1995). A cys115ser variant was reported by Lin *et al.* (1996), who named the alleles *ALDH9A1*1* and **2* (any differences in enzymatic activity are not yet known). *ALDH1B1* (originally designated *ALDH5*; Hsu & Chang, 1991) is unique among the *ALDH* genes as it lacks introns. Its enzyme is closely related to ALDH2 (72% sequence similarity) and its N-terminus may be a mitochondrial leader sequence. The *ALDH1B1* gene is polymorphic at two

different residues: valine or alanine at position 69 and leucine or arginine at position 90 of the protein (Hsu & Chang, 1991; Sherman *et al.*, 1993a), but it is not known if these substitutions alter its enzymatic properties. The highest levels of *ALDH1B1* mRNA are expressed in liver, kidney and skeletal muscle (Stewart *et al.*, 1996a).

ALDH3A1 and ALDH4A1 are widely expressed, but have low affinity for aliphatic aldehydes and higher affinity for aromatic aldehyde substrates. The ALDH3 family includes the cytosolic, tetrachlorodibenzo-*para*-dioxin-inducible ALDH, the hepatoma-associated ALDH, and the corneal and gastric ALDH3 (Vasiliou *et al.*, 1993, 2000, 2004). The gastric form may oxidize acetaldehyde generated during gastric metabolism of ethanol. ALDH4 has been identified as glutamic γ-semialdehyde dehydrogenase (or Δ-1-pyrroline-5-carboxylate dehydrogenase); ALDH6A1 is methylmalonyl semialdehyde dehydrogenase (Kedishvili *et al.*, 1992); the functions of ALDH7 and ALDH8 are not yet known (Hsu *et al.*, 1995; Fong *et al.*, 2006).

The *ALDH1A1* gene has been cloned (Hsu *et al.*, 1989), and the promoter has been studied in transfection and DNA-binding assays. A minimal promoter was shown to bind nuclear factor (NF)-Y/CP1 and octamer factors (Yanagawa *et al.*, 1995). Two polymorphisms, a 17 base-pair deletion (−416/-432; *ALDH1A1*2*) and a 3 base-pair insertion (–524; *ALDH1A1*3*), were discovered in the *ALDH1A1* promoter. *ALDH1A1*2* was observed at frequencies of 0.035, 0.023, 0.023 and 0.012 in Asian, Caucasian, Jewish and African-American populations, respectively. *ALDH1A1*3* was observed only in the African-American population at a frequency of 0.029 (Spence *et al.*, 2003). In an African-American population, a significant association was observed between the *ALDH1A1*3* allele and patients with alcoholism (p=0.03); a trend was also observed that the *ALDH1A1*2* allele was more frequent in the alcoholic group (p=0.12). In Asian populations, *ALDH1A1*3* was not observed and *ALDH1A1*2* yielded no significant association with alcoholism, when controlling for the *ALDH2*2* genotype (Spence *et al.*, 2003). In a population of Indians in Southwest California, it was suggested that the *ALDH1A1*2* allele may be associated with a protective effect against the development of alcohol use disorders (Ehlers *et al.*, 2004). In inhabitants of Trinidad and Tobago of East Indian and African descent, the *ALDH1A1*2* allele was found to be associated with increased risk for the development of alcoholism in those of Indian origin (Moore *et al.*, 2007).

The importance of ALDH2 in ethanol oxidation is emphasized by the alcohol flush reaction (Goedde *et al.*, 1979; Harada *et al.*, 1981). Alcohol-induced facial flushing is common in Japanese, Chinese and Koreans, while these reactions are rare among Caucasians (Wolff, 1972). Flushing correlates with the accumulation of acetaldehyde (Mizoi *et al.*, 1979). In non-flushers, drinking alcoholic beverages elicited a small increase in acetaldehyde levels (to 3–5 μM); in flushers, the levels were variable, but could exceed 80 μM (Enomoto *et al.*, 1991a,b). The activity of ALDH (ALDH1 and ALDH2) in hair roots was examined in individuals who reported flushing (associated with ALDH1-deficiency characterized by electrophoretic assays); about 40% of Japanese had ALDH2 activity (Harada *et al.*, 1982), and most flushed when they

drank, which indicates that ALDH2 plays a crucial role in maintaining low levels of acetaldehyde during ethanol oxidation (Harada *et al*., 1983). The *ALDH2*2* allele deficiency was reported in South American and North American Indians (Novoradovsky *et al*., 1995a) and ALDH2 enzyme deficiency was shown in Chachi Indians of Ecuador (Novoradovsky *et al*., 1995b). However, a new allele, *ALDH2*3*, was detected in North American Indians. The mutation responsible for the deficiency is a G→A substitution that results in a glutamate to lysine substitution at position 487 of the enzyme (Yoshida *et al*., 1984; Crabb *et al*., 1989). The normal allele is *ALDH2*1* and the mutant allele is designated *ALDH2*2*. The *ALDH2*2* heterozygotes, as well as homozygotes, are ALDH2-deficient (Crabb *et al*., 1989), but the homozygotes have much higher acetaldehyde levels after they drink alcoholic beverages than the heterozygotes; consistent with this, the heterozygotes have residual low-K_m ALDH activity in liver biopsies (Enomoto *et al*., 1991a). It is estimated that about 30% of total liver ALDH activity is ALDH2 and 70% is contributed by other forms (ALDH1A1, ALDH9A1 and possibly ALDH1B1) when assayed with 200 μM acetaldehyde (Yao *et al*., 1997).

Studies on the effect of ALDH2-deficiency on ethanol elimination rates are limited by the severity of the flushing reaction. Early studies did not show a difference in ethanol elimination rates between flushers and non-flushers (Mizoi *et al*., 1979; Inoue *et al*., 1984), but a subsequent study detected reduced rates of ethanol elimination in individuals with ALDH2-deficiency when the subjects were stratified by *ADH* genotype (Mizoi *et al*., 1994).

A mutation in the *ALDH2* promoter was simultaneously reported by Harada *et al.* (1999) and Chou *et al.* (1999). This A/G variant occurs at about −360 base-pair distance from the hepatocyte nuclear factor 4 (HNF4) binding site. The A allele is less active than the G allele in reporter-gene transfection assays (Chou *et al*., 1999), and is less common in alcoholics with active ALDH2 (Harada *et al*., 1999). These variants have been found in all ethnic groups. There is also one additional reported variant, designated $ALDH2^{2Taiwan}$, which involves a glutamate to lysine substitution at position 479 in addition to the *ALDH2*2* variant (Novoradovsky *et al*., 1995a). Whether this variant alters the dominant negative effect of *ALDH2*2* is unknown.

Tissue distribution

ALDH1A1 and *ALDH2* mRNAs are expressed in a variety of human tissues in addition to the liver (Stewart *et al*., 1996a); *ALDH2* mRNA was particularly abundant in the kidney, muscle and heart. Low levels of *ALDH1A1* and *ALDH2* mRNAs were found in the placenta, brain and pancreas; these are obviously target organs for alcoholic pathology, consistent with the hypothesis that the presence of ALDHs is protective against the toxicity of acetaldehyde (Table 4.2 and Figure 4.2). Colonic and oesophageal mucosae express low levels of low-K_m ALDH activity (Yin *et al*., 1993, 1994). In the colon, the activity of low-K_m ALDH was similar whether the individual was ALDH2-sufficient or -deficient, which supports the notion that the major enzyme present was ALDH1A1. In the oesophagus, overall low-K_m ALDH activity was low and was predominantly attributable to ALDH1A1. Morita *et al.* (2005) reported the

presence of immunoreactive ALDH2 in the oesophagus of moderate-to-heavy alcoholic beverage drinkers, but no or low expression of ALDH2 in the oesophagus of non-drinkers or light drinkers, and speculated that the difference was related to *ALDH2*2* status; however, this allele has not been associated with the absence of immunoreactive ALDH2 protein in the past. Breast epithelium is reported to express ALDH1A1 and ALDH3 (Sreerama & Sladek, 1997). There are no reports of ALDH2 enzyme activity in the breast, but the expressed sequence tag database suggests that *ALDH2* and *ALDH1B1* transcripts are present (Figure 4.2). Examination of the GEO profiles database (at: http://www.ncbi.nih.gov/geo) suggests that normal breast tissue may express *ALDH1A1* and *ALDH2* mRNA.

4.2.2 *Experimental systems*

(a) Ethanol

(i) *ADH pathway*

Several classes of *Adh* genes are expressed in animals: class VI *Adh* was reported in deer-mouse and rat liver (Höög & Brandt, 1995); and class VII *Adh* was cloned from chicken (Kedishvili *et al.*, 1997), but the human homologues of these have not been found.

Tissue distribution

As in humans, ADHs are expressed in a variety of tissues in rats and mice. High levels of class I ADH activity were found in the liver, lung, small intestine, colon, duodenum, stomach, kidney, testis, epididymis and uterus, and mRNA was detectable in most tissues of rats (Estonius *et al.*, 1993; Table 4.3). Cytosolic ADH has been found in the parotid gland of rats, and chronic alcoholic beverage use was associated with parotid steatosis (Maier *et al.*, 1986). Class IV ADH is found in the blood vessels of rats (Allali-Hassani *et al.*, 1997). ADH activity with octanol was reported to be present in numerous epithelial tissues, which may reflect the presence of either class II or class IV *Adh* (Svensson *et al.*, 1999; Crosas *et al.*, 2000). Haber *et al.* (1998) reported that pancreatic acinar cells metabolize ethanol via class III *Adh* (see Table 4.3) (Julià *et al.*, 1987; Boleda *et al.*, 1989).

Variation in expression

Fasting reduces ADH activity in rats (Bosron *et al.*, 1984), which correlates with ethanol elimination rates (Lumeng *et al.*, 1979), whereas growth hormone induces rat ADH activity (Mezey & Potter, 1979). Chronic ethanol consumption can affect the expression of Adh: ethanol increased hepatic ADH activity in male rats by reducing testosterone levels (Rachamin *et al.*, 1980). The amount of ethanol consumed from conventional liquid diets did not alter liver ADH activity, whereas higher doses achieved by intragastric infusion of ethanol induced this activity. In rats, class I *Adh* mRNA and enzyme activity are inducible by administration of high levels of ethanol by gastric infusion. This leads to cyclic changes in blood ethanol concentrations despite continuous infusion of ethanol. Regulation of rat hepatic *Adh* gene expression by ethanol has

Figure 4.2. Tissue distribution of aldehyde dehydrogenase (*ALDH*) transcripts reflected by the abundance of expressed sequence tags

Tissue	*ALDH1A1*	*ALDH2*	*ALDH1B1*	*ALDH9A1*
Adipose tissue	360	504	72	432
Adrenal gland	1506	384	29	324
Blood	123	53	23	169
Bone	27	55	55	41
Bone marrow	306	0	20	102
Brain	360	119	22	185
Cervix	103	20	0	228
Colon	272	198	59	59
Connective tissue	326	34	6	217
Eye	231	115	14	106
Heart	178	133	33	156
Kidney	648	84	75	338
Larynx	65	65	0	0
Liver	1439	376	14	138
Lung	437	138	8	115
Lymph	0	134	22	22
Lymph node	0	83	10	20
Mammary gland	81	35	23	245

Figure 4.2 (contd)

Mouth	477		57		28		159	
Muscle	78		34		0		95	
Nerve	119		239		0		119	
Oesophagus	156		0		104		156	
Ovary	65		150		0		28	
Pancreas	182		91		9		54	
Pharynx	351		43		0		329	
Placenta	84		40		3		90	
Prostate	135		65		35		175	
Salivary gland	48		0		0		97	
Skin	217		95		74		127	
Small intestine	5103		112		22		474	
Spleen	813		18		18		302	
Stomach	1047		264		48		97	
Testis	733		60		37		266	
Thymus	193		0		0		296	
Thyroid	90		200		54		345	
Tonsil	0		116		0		0	
Trachea	2784		0		20		329	
Urinary bladder	725		65		0		32	
Uterus	928		58		62		150	
Vascular	533		59		19		197	

The number given for each tissue is the abundance of the expressed sequence tag in terms of transcripts/million.
This Figure is compiled from information publicly available at the National Center for Biotechnology Information (NCBI) (see http://www.ncbi.nlm.nih.gov/unigene)

Table 4.3 Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) enzyme activity and mRNA distribution in rats

Enzyme	Activity	mRNA	References
Class I (ADH3)	Liver, lung, small intestine, colon, kidney, testis, epididymis, uterus	Most tissues in varying amounts	Estonius *et al.* (1993); Boleda *et al.* (1989)
Class II (ADH1)	Eye, ear canal, nasal and buccal mucosa, trachea, lung, tongue, oesophagus, stomach, rectum, vagina; lower in intestine, adrenals, colon, testis, epidiymis, ovary, uterus, urinary bladder, penis, skin	Liver, duodenum, kidney, stomach, spleen, testis	Estonius *et al.* (1993); Boleda *et al.* (1989) Note: Reported studies probably detected both class II and class IV ADH in various tissues, due to overlapping substrate specificities
Class III (ADH2)	Ubiquitous	All tissues	Estonius *et al.* (1993); Boleda *et al.* (1989)
Class IV (σ-ADH)	Skin, ears, eye, nasal and buccal mucosa, tongue, vagina, oesophagus, penis, rectum, blood vessels	Not examined	
ALDH1A1	Liver	Not examined	
ALDH2	Liver, vascular tissue	Not examined	Sydow *et al*. (2004)
ALDH1B1	Liver	Not examined	
ALDH9A1	Liver	Not examined	Kurys *et al.* (1989)

Most of the ADH activity data are from Julià *et al.* (1987); Boleda *et al*. (1989); Allali-Hassani *et al.* (1997) (blood vessels).

been proposed to be due to induction of the transcription factor CCAAT enhancer-binding protein β (C/EBPβ) and suppression of C/EBPγ, a truncated, inhibitory form of C/EBPβ called liver inhibitory protein (He *et al*., 2002), and of sterol regulatory element-binding protein-1 (SREBP-1) (He *et al*., 2004). In addition, chronic intragastric infusion of ethanol increases portal vein endotoxin, which can induce *Adh* mRNA via increased binding of upstream stimulatory factor to the *Adh* promoter (Potter *et al*., 2003).

Role of substrate and product concentrations in controlling ADH activity

Modelling of ethanol oxidation in rat liver indicated that ADH activity was controlled by the total activity of the ADH enzyme as well as by product inhibition by NADH and acetaldehyde; thus ADH operates below its V_{max} at steady-state (Crabb *et al*., 1983). Liver NADH levels are elevated during ethanol oxidation because the first enzyme in the malate–aspartate shuttle, malate dehydrogenase, has a high K_m for NADH, and thus is more active as the level of NADH rises. The high level of NADH does not limit the rate of the shuttle or mitochondrial re-oxidation of NADH, as had been suggested (Crow *et al*., 1982). Flux through the pathway is also dependent on the total activity of

ADH. Reduction in total ADH activity (as occurs during fasting) reduced the ability of the liver to oxidize ethanol in rats. In contrast, increases in ADH activity did not increase the metabolic rate proportionally (Crabb *et al.*, 1983). Metabolism of ethanol can be acutely increased when a large intragastric dose of ethanol (5 g/kg bw) is given to rats. This swift increase in ethanol metabolism is dependent upon activation of the sympathic nervous system, activation of Kupffer cells, depletion of liver glycogen, increased plasma fatty acids and increased provision of cofactors for ADH (NAD^+) and catalase (hydrogen peroxide). This phenomenon may contribute to the hepatotoxicity of heavy alcoholic beverage consumption (Bradford & Rusyn, 2005).

Regulation of *Adh* gene expression *in vitro*

The *Adh1* promoters are all active in the liver. Transfection studies and experiments using nuclear extracts have shown that the *Adh* promoters interact with ubiquitous transcription factors (e.g. TATAA binding factors, upstream stimulatory factor, CCAAT transcription factor/NF-1 and specificity protein 1-like factors), as well as tissue-specific factors (e.g. HNF-1, D box-binding protein and C/EBPα and β; reviewed by Edenberg, 2000). The *Adh5* (class III *Adh*) and *Adh7* (class IV *Adh*) promoters lack TATAA boxes (Edenberg, 2000). The *Adh5* promoter is GC rich, which is a characteristic of housekeeping genes and consistent with its ubiquitous expression. Binding sites for thyroid hormone, retinoic acid and glucocorticoid receptors have been identified in the upstream regions of Class I *Adh* genes. In rats, hypothyroidism increased and hyperthyroidism decreased ADH activity in liver and kidney. It is not clear whether these effects occur at the level of transcription or translation, on the half-life of the ADH protein, or a combination of these (Dipple *et al.*, 1993). Growth hormone increased ADH activity in rats and cultured hepatocytes, while thyroid hormones decreased it (Potter *et al.*, 1993); androgens increased ADH activity in mouse kidney and reduced it in the adrenal glands (in Edenberg, 2000).

No post-translational modifications of the ADH enzyme have been recognized. However, in an in-vitro study peroxynitrite oxidized the active site of yeast ADH, which caused disulfide-bond formation and release of zinc, which inactivated the enzyme (Daiber *et al.* 2002); this could lead to inactivation of ADH at sites where nitric oxide is formed. Whether this is physiologically relevant remains to be shown.

(ii) *Microsomal ethanol-oxidation pathway*

Control of expression of CYP2E1

The human *CYP2E1* gene spans 11 kb, contains 9 exons and a typical TATAA box. HNF1α is critical for its expression (Liu & Gonzalez, 1995).

Expression is also controlled both at the level of mRNA (high concentrations of ethanol can induce transcription of the *CYP2E1* gene; Takahashi *et al.*, 1993) and by stabilization of the protein, as observed for ethanol, acetone and pyrazole derivatives (Takahashi *et al.*, 1993; Lieber, 2004a,b). Other data suggest that additional signals may affect its expression. For instance, CYP2E1 can be induced by interleukin (IL)-4 in human hepatoma cells (Lagadic-Gossmann *et al.*, 2000) and by phorbol ester and other

cellular stress factors, such as ischaemic injury in astrocytes (Tindberg, 2003). Insulin reduced the expression of CYP2E1 post-transcriptionally by destabilizing its mRNA (Woodcroft *et al.*, 2002). Castro *et al.* (2006) reported ethanol-inducible, microsomal ethanol-oxidizing activity in the rat mammary gland. In young female Sprague-Dawley rats, ethanol fed in a liquid diet resulted in a 30–50% increase in ethanol metabolism in mammary tissue extracts. CYP2E1 is also expressed in the kidney (Ronis *et al.*, 1991), lung (Yang *et al.*, 1991), rat colon mucosa (Hakkak *et al.*, 1996), brain (Tindberg & Ingelman-Sundberg, 1996), duodenum and jejunum (Shimizu *et al.*, 1990). After chronic feeding of ethanol, immunoreactive CYP2E1 was found in the buccal mucosa, oesophagus, tongue, forestomach and proximal colon of rats (Shimizu *et al.*, 1990).

CYP2E1 is reported to be a substrate for cAMP-dependent protein kinase A. Phosphorylation of a serine residue inactivates the enzyme (Oesch-Bartlomowicz *et al.*, 1998). Whether this plays a physiological role in controlling the activity of this enzyme is not clear, although, under several conditions in which CYP2E1 activity is low (fasting, diabetes), hepatic protein kinase A activity is high.

(iii) *Oxidation by catalase*

The activity of catalase depends upon the availability of hydrogen peroxide. When fatty acids were perfused through rat liver, peroxisomal β-oxidation generated hydrogen peroxide and stimulated ethanol oxidation. This raises the possibility that, under conditions of increased fatty acid oxidation (fasting, high fat diet) or oxidant stress (and production of hydrogen peroxide), catalase-mediated ethanol oxidation may be increased. Chronic ethanol feeding was reported to increase catalase activity (Orellana *et al.*, 1998). In ADH-deficient deermice, ethanol and methanol oxidation were highly sensitive to inhibition by the catalase inhibitor, aminotriazole (Bradford *et al.*, 1993).

Regulation of catalase gene expression *in vitro*

Little is known regarding transcriptional control of catalase expression in mammalian cells. The rat catalase gene is a single-copy gene that spans 33 kb. The promoter region lacks a TATAA box and an initiator consensus sequence, contains multiple CCAAT boxes and GC boxes, and contains multiple transcription initiation sites, consistent with its housekeeping function (Nakashima *et al.*, 1989). The rat catalase promoter contains a peroxisome proliferator-responsive element (Girnun *et al.*, 2002) and can be induced by peroxisome proliferators. In cells exposed to hydrogen peroxide, the non-receptor protein tyrosine kinases, Abl and Arg, associate with catalase and can activate it by phosphorylating two tyrosine residues. However, at higher concentrations of hydrogen peroxide, phosphorylation of these residues can stimulate ubiquitination and proteasomal degradation of the enzyme (Cao *et al.*, 2003).

(b) Acetaldehyde

Aldehyde dehydrogenase

Ethanol does not induce ALDH2 expression. Dietary restriction and protein deficiency, both common in human alcoholic patients, reduced ALDH2 activity in rats.

A recent report (Moon *et al.*, 2006) suggested that ALDH2 may be inhibited during chronic ethanol feeding through oxidant stress, which leads to the formation of nitric oxide and nitrosylation of the active cysteine site of ALDH2. This was not recognized in earlier studies, partly because thiol reagents such as dithiothreitol, which is used in the preparation of tissue and cell homogenates, reverse the formation of the nitrosylated enzyme. ALDHs are widely distributed in animal tissues (Oyama *et al.*, 2005) (Table 4.3 and Figure 4.2). ALDH was found in the nasal respiratory epithelium (the ciliated epithelial cells) of rats, although the olfactory epithelium lacked ALDH activity. There was low activity in the trachea but the Clara cells of the lower bronchioles exhibited high activity (Bogdanffy *et al.*, 1986). However, it is unknown which class of ALDH this represents. ALDH2 is important in the bioactivation of nitrate vasodilators such as glyceryl trinitrate; the enzyme is present in the muscle layer of the blood vessels (Sydow *et al.*, 2004).

Because of the influence of the *ALDH2* genotype on alcoholic beverage consumption in humans, variations in rat ALDH2 enzyme have been investigated. Several coding region polymorphisms exist. Rats that have a preference for ethanol (ethanol-preferring) express an ALDH2 with glutamine at position 67 (ALDH2Gln), while rats that do not (non-preferring) express an ALDH2 with arginine at that position (ALDH2Arg). However, the enzymatic properties of the purified enzymes are similar, and the different isozymes were not associated with high or low ethanol intake in the F_2 generations of intercrosses of the ethanol-preferring and non-preferring rats (Carr *et al.*, 1995). These variants are also found in rats that accept (ethanol-accepting) ethanol and those that do not (non-accepting). Of interest, the non-accepting rats had higher blood acetaldehyde levels after administration of ethanol; however, rat strains did not differ in the frequencies of the *Aldh2Arg* and *Aldh2Gln* alleles (Koivisto *et al.*, 1993). While there was no reported difference in acetaldehyde levels after ethanol consumption between UChA (low ethanol-drinking) and UChB (high ethanol-drinking) rat strains, 94% of the UChA rats had the *Aldh2Arg* allele, while the UChB rats had either the Sprague-Dawley allele *Aldh2Gln* or the *Aldh2Arg* plus an additional substitution of lysine for glutamine at position 479, i.e. *Aldh2Lys*. Ethanol-drinking patterns in these rats correlated well with the *Aldh2* genotype (Sapag *et al.*, 2003). The K_m for NAD^+ was 4- to 5-fold higher for the ALDH2Arg enzyme than for ALDH2Gln or ALDHLys. It appears that variation in ALDH2 activity in rats may affect their ethanol preference, and that there may be strain differences in acetaldehyde metabolism that are relevant to studies on the carcinogenicity of ethanol and acetaldehyde.

Transgenic mice that lack ALDH2 activity have been created by knockout technology (Isse *et al.*, 2002). These mice have reduced ethanol preference and, when exposed to higher doses of ethanol by gavage, have elevated acetaldehyde levels in the blood, liver and brain (Isse *et al.*, 2005). These animals have been used for toxicological studies of ethanol and acetaldehyde (see Section 4.5).

In-vitro studies

The human and rat recombinant ALDH2*2 enzymes expressed in *Escherichia coli* have a much higher K_m for NAD^+ and a lower V_{max} compared with the wild-type enzyme (Farrés *et al.*, 1994a). Xiao *et al.* (1995, 1996) expressed the two human *ALDH2* alleles in tissue cultures of Hela and CV-1 cells, which do not naturally express ALDH2. *ALDH2*1* directed expression of an active low-K_m ALDH2. The *ALDH2*2* allele directed expression of a functionally inactive but immunoreactive protein (ALDH2Lys). Transduction of *ALDH2*2* into *ALDH2*1*-expressing cells (*Aldh2Glu*) reduced the ALDH2 activity substantially, which suggests that only enzymes with tetramers that contain either three or four wild-type subunits are active (Xiao *et al.*, 1995); the *ALDH2*2*-containing tetramers were less stable and further reduced the activity of heterotetramers (Xiao *et al.*, 1996). The X-ray crystal structure of ALDH2 showed that the mutation occurs in a region of the protein that is involved in subunit–subunit interaction (Steinmetz *et al.*, 1997). Introduction of a positive charge at position 487 (Glu 487 Lys) disrupts ionic bonds with arginines that are normally neutralized by the glutamate; this may suffice to inactivate the adjacent subunits and explain the dominance of the mutation.

The *ALDH2* gene has been studied extensively. It has no TATAA box (Hsu *et al.*, 1988); similarly to *ALDH1A1*, it has a binding site for the ubiquitous NF-Y/CCAAT protein 1 (NF-Y/CP1) near the transcription start site (Stewart *et al.*, 1996b). Pinaire *et al.* (1999) found that, upstream from the CCAAT box, there is a promoter site bound by hepatocyte nuclear factor 4 (HNF-4) and retinoid X receptor, which activate expression, while apolipoprotein A regulatory protein-1, chicken ovalbumin upstream promoter-transcription factor and peroxisome proliferator-activated receptor δ oppose this activation. It is probable that this site integrates the effects of several different transcription factors in different tissues and this regulatory mechanism may explain the tissue specificity of expression.

4.3 Genetic susceptibility

4.3.1 *Humans*

(a) Genes encoding enzymes involved in alcohol metabolism

(i) *ADH-1B*

ADH1B (previously called *ADH2*) is polymorphic, and its superactive *ADH1B*2* allele is highly prevalent among East Asians (i.e. 54–96%; Goedde *et al.*, 1992), but relatively rare among Caucasians (i.e. 1–23%). The less active *ADH1B*1* is a risk factor for alcoholism in both East Asians and Caucasians (Zintzaras *et al.*, 2006). *ADH1B*1/*1* carriers showed an increased risk for upper aerodigestive tract cancer (odds ratio, 1.6–8.2 versus *ADH1B*1/*2* and *ADH1B*2/*2* carriers) in eight case–control studies of Japanese, Taiwanese, Thai and central European populations (reviewed in Yokoyama

& Omori, 2005; see Table 4.4) and in a prospective cohort study in cancer-free Japanese alcoholics (hazard ratio, 2.0; Yokoyama *et al.*, 2006b; Table 4.5), but there was no increased risk found in two Japanese studies, including a study of women that involved a small number of cases (Yang *et al.*, 2005; Yokoyama *et al.*, 2006a).

Two Japanese case–control studies reported overall negative results for an association between *ADH1B* genotype and hepatocellular carcinoma (Takeshita *et al.*, 2000a; Sakamoto *et al.*, 2006; Table 4.6). One Japanese case–control study reported an *ADH1B*1*-associated increased risk for colorectal cancer (odds ratio, 1.9 for **1/*1*; 1.4 for **1/*2*; 1.0 for **2/*2*; Matsuo *et al.*, 2006a). A statistically significant increase in the risk for colorectal cancer was observed for the *ADH1B*1/*1* genotype compared with the *ADH1B*2/*2* genotype, with adjustment for alcoholic beverage intake and other factors. The interaction with alcoholic beverage intake was also examined for the composite genotypes of *ADH1B* and *ALDH2* (see below). A case–control study in Spain reported a statistically non-significant decrease in the risk for the *ADH1B*2/*2* versus *ADH1B*1/*1* genotype (Landi *et al.*, 2005; Table 4.6).

In a large German study (Lilla *et al.*, 2005), a decreased risk for breast cancer for high alcoholic beverage intake (≥12 g ethanol/day versus no intake) was observed in women with the *ADH1B*2* allele, whereas no such association was found in women with the *ADH1B*1/*1* genotype (interaction p=0.05).

ADH1B*1/*1 has an approximately 40 times lower V_{max} than ADH1B*2/*2 (reviewed in Bosron & Li, 1986). Although the *ADH1B* genotype did not affect peak blood acetaldehyde concentration after light alcoholic beverage consumption (Mizoi *et al.*, 1994), a clamping technique with intravenous infusion of ethanol has shown modestly but significantly lower ethanol elimination rates among men who have *ADH1B*1/*1* than among those who have the *ADH1B*2* allele (Neumark *et al.*, 2004). After moderate-to-heavy alcoholic beverage consumption, ethanol may linger in the blood and saliva for longer periods in *ADH1B*1/*1* carriers than in carriers of other genotypes, and lead to prolonged exposure to acetaldehyde in the upper aerodigestive tract as a result of acetaldehyde production by oral bacterial and mucosal ADHs (Homann *et al.*, 2000a).

Individuals with a combination of the *ALDH2*1/*2* and *ADH1B*1/*1* genotypes tend not to experience alcoholic flushing after oral intake of small amounts of alcoholic beverage (Takeshita *et al.*, 1996; Yokoyama *et al.*, 2003), and the diminished intensity of the aversive flushing response among *ALDH2* heterozygotes has been found to be positively associated with higher daily alcoholic beverage consumption (Yokoyama *et al.*, 2003). Japanese who have the *ADH1B*1/*1* genotype are at high risk for heavy drinking (Matsuo *et al.*, 2006b) and for developing alcoholism. Japanese alcoholics who have the *ADH1B*1/*1* genotype are more prone to binge drinking and the withdrawal syndrome earlier in life than those with other genotypes (reviewed in Eriksson *et al.*, 2001). Such *ADH1B*1/*1*-facilitated drinking patterns may affect the risk for alcohol-related cancer.

[The Working Group noted that the available genetic epidemiological data suggest a positive association between *ADH1B*1/*1* and upper aerodigestive tract cancer, but

Table 4.4 Case–control studies of *ALDH2*, *ADH1B* and *ADH1C* genotype-associated risks for cancer (upper aerodigestive tract)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Yokoyama *et al*. (1996), Kanagawa, Chiba, Japan, 1991–95	Oesophageal cancer	29 male daily drinkers from Ichikawa General Hospital, 40 alcoholic men from Kurihama National Hospital, aged 44–80 years, Japanese	28 male daily drinkers recruited from the staff Kurihama National Hospital and their acquaintances and 55 alcoholic men from the hospital, aged 41–77 years, Japanese	Structured interview	*ALDH2* Daily drinkers Alcoholics	 12.1 (3.4–42.8) 7.6 (2.8–20.7)	None	
Hori *et al*. (1997), Tokyo, Japan	Oesophageal squamous-cell carcinoma	94 (78 men) from Tokyo Medical and Dental University, Japanese	70 new healthy subjects (43 men) plus 60 healthy men in an other study, Japanese	Not described	Overall *ALDH2* *ADH1B*	 4.4 (2.5–7.7) 6.2 (2.6–14.7)	None	
Yokoyama *et al*. (1998a), Kanagawa, Japan, 1987–97	Oesophageal cancer	87 alcoholic men (71 incident cases, 16 prevalent cases) from Kurihama National Hospital, aged 55±7 years, Japanese	487 cancer-free alcoholic men from the hospital, aged 53±8 years, Japanese	Structured interview	*ALDH2* Alcoholics	 12.5 (7.2–21.6)	Age, drinking, smoking	Because the differences in odds ratio between the incident cases and the prevalent cases were slight, the cases were combined.
	Oropharyngo-laryngeal cancer	34 alcoholic men (19 incident cases, 15 prevalent cases) from the hospital, aged 55±8 years, Japanese				11.1 (5.1–24.4)		
Katoh *et al*. (1999), Kitakyushu, Japan, 1992–98	Oral squamous-cell carcinoma	92 (56 men) from UOEH Hospital, aged 62±12 years, Japanese	147 hospital-based (91 men) from another hospital in Kitakyushu, aged 70±11 years, Japanese	Interview	Overall *ALDH2*	 1.2 (0.7–2.1)	Age, sex, drinking	Alcoholic beverage drinking not significantly associated with the risk for oral cancer

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Tanabe *et al*. (1999), Hokkaido, Japan, 1994–97	Oesophageal squamous-cell carcinoma	19 patients (17 men) from Asahikawa Medical College Hospital, aged 64±10 years, Japanese	25 patients with head and neck squamous-cell carcinoma (21 men) from the hospital, aged 61±10 years, Japanese	Questionnaire	*ALDH2*	Significantly increased (p<0.009)	None	Alcohol consumption and smoking did not differ between the cases and controls.
Chao *et al*. (2000), Taipei, Taiwan, China, 1997–99	Oesophageal cancer	59 alcoholic men (56 squamous-cell carcinoma , 3 adenocarcinoma) from Tri-Service General Hospital and Veterans General Hospital, aged 65±12 years, Chinese	222 alcoholics (208 men; pancreatitis in 87, cirrhosis in 116, both in 19) from the hospitals, aged 41±11–51±13 years, Chinese	Not described	Alcoholics *ALDH2* *ADH1B*	 Significantly increased (p<0.001) Significantly increased (p<0.025)	None	
Nomura *et al*. (2000), Chiba, Japan, 1996–98	Oral squamous-cell carcinoma	191 (121 men) from Tokyo Dental College, aged 24–94 years, Japanese	121 hospital-based (69 men), aged 40–70 years, Japanese	Not described	Habitual drinkers *ALDH2*	 2.9 (1.1–7.8)	None	Habitual drinking increased the risk for oral cancer (odds ratio, 3.9 [2.4–6.3]).
Matsuo *et al*. (2001), Aichi, Japan, 1984–2000	Oesophageal cancer	102 (86 men) from Aichi Cancer Center, aged 40–76 years, Japanese	241 hospital-based (118 men) from the Center, aged 39–69 years, Japanese	Self-administered questionnaire	*ALDH2* Heavy drinkers (75 mL ethanol/day, ≥5 days/week) Others	 16.4 (4.4–61.2) 1.7 (0.8–3.6)	Age, sex, drinking, smoking	

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Yokoyama *et al.* (2001), Kanagawa, Japan, 1993–2000	Oesophageal squamous-cell carcinoma	112 alcoholic men from Kurihama National Hospital, aged 56±7 years, Japanese	526 cancer-free alcoholic men from the hospital, aged 53±8 years, Japanese	Structured interview	Alcoholics *ALDH2* *ADH1B*	 13.5 (8.1–22.6) 2.6 (1.6–4.3)	Age, drinking, smoking, *ALDH2* and *ADH1B* genotypes	
	Oropharyngo-laryngeal squamous-cell carcinoma	33 alcoholic men from the hospital, aged 54±8 years, Japanese			*ALDH2* *ADH1B*	18.5 (7.7–44.5) 6.7 (2.8–15.9)		Odds ratios for oral/oro-pharyngeal squamous-cell carcinoma, 20.8 (95% CI; 6.6–65.5); and for hypo-pharyngeal/ epilaryngeal squamous-cell carcinoma, 28.9 (95% CI; 8.7–96.6)
Yokoyama *et al.* (2001) (contd)	Multiple primary oesophageal squamous-cell carcinoma	45 alcoholic men with multiple primary intraoesophageal squamous-cell carcinoma	67 alcoholic men with solitary intraoesophageal squamous-cell carcinoma		*ALDH2* *ADH1B*	3.4 (1.5–7.9) 0.8 (0.3–1.7)		
	Multi-organ primary cancer with oesophageal squamous-cell carcinoma	22 alcoholic men with both oesophageal squamous-cell carcinoma and either oropharyngo-laryngeal squamous-cell carcinoma or gastric adenocarcinoma	90 alcoholic men with oesophageal squamous-cell carcinoma alone		*ALDH2* *ADH1B*	4.0 (1.2–13) 1.2 (0.4–3.4)		

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Boonyaphiphat *et al*. (2002), Songkhla, Thailand, 1997–2000	Oesophageal squamous-cell carcinoma	202 (172 men) from Songklanagarind Hospital, aged 64±10 years, Thai	261 hospital-based (225 men) from the hospital who had no alcohol- or tobacco-related diseases, aged 65±12 years; matched by age, sex, ethnicity	Structured interview	Overall *ALDH2* *ADH1B* *ALDH2*1/*1* 0 ≤60 g/day >60 g/day *ALDH2*1/*2* 0 ≤60 g/day >60 g/day *ADH1B*1/*1* 0 ≤60 g/day >60 g/day *ADH1B*1/*2* 0 ≤60 g/day >60 g/day	 1.6 (0.9–2.8) 1.6 (1.01–2.4) Interaction p=0.064 1 2.2 (1.1–4.2) 5.3 (2.7–10.3) 1.6 (0.7–3.7) 2.5 (0.9–7.5) 10.8 (3.4–34.7) Interaction p=0.031 0.9 (0.4–1.9) 2.3 (1.1–5.1) 11.5 (5.2–25.5) 1 2.0 (1.0–4.1) 3.4 (1.5–7.0)	Age, sex, smoking, betel chewing, (drinking, *ALDH2* and *ADH1B* genotypes for overall)	Unlike Japanese and Chinese studies, frequency of inactive *ALDH2* is low in Thais: 20% in cases, 18% in controls.
Itoga *et al*. (2002), Chiba, Japan	Oesophageal cancer	82 men (65 habitual drinkers) from Chiba University Hospital, aged 65±10 years, Japanese	192 healthy controls (151 habitual drinkers), aged 51±9 years, Japanese	Questionnaire	Habitual drinkers *ALDH2*	 4.9 (p<0.0001)	None	
Yokoyama *et al*. (2002a), Tokyo, Chiba, Japan, 1998–99	Multiple primary cancer with oesophageal squamous-cell carcinoma	26 men from National Cancer Center Hospital and National Cancer Center Hospital East, aged 61±8 years, Japanese	48 men with solitary intra-oesophageal squamous-cell carcinoma alone from the hospitals, aged 63±9 years, Japanese	Structured questionnaire	Overall *ALDH2*	 5.3 (1.1–51.1) **2/*2* or **1/*2* versus **1/*1*	Age, sex, drinking, smoking	Multiple cancers included both multi-organ cancer and multiple intra-oesophageal squamous-cell carcinoma
	Multi-organ primary cancer with head and neck squamous-cell carcinoma	17 men from National Cancer Center Hospital and National Cancer Center Hospital East, aged 61±10 years; Japanese	29 men with solitary head and neck squamous-cell carcinoma alone from the hospitals, aged 61±13 years, Japanese		*ALDH2*	7.4 (1.3–80.1) **2/*2* or **1/*2* versus **1/*1*		

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Yokoyama *et al*. (2002b), Tokyo, Chiba, Kanagawa, Osaka, Japan, 2000–01	Oesophageal squamous-cell carcinoma	234 men from Tokyo, Chiba, Kanagawa and Osaka hospitals, aged 40–79 years, Japanese; response rate, 99%	634 cancer-free men who underwent an annual medical check-up at one of two Tokyo clinics, aged 40–79 years; Japanese; response rate, 86%	Structured questionnaire	Overall *ALDH2* *ADH1B* *ADH1C* *ALDH2*1/*1* <22 g/week 22–197 g/week 198–395 g/week ≥396 g/week Former drinker *ALDH2*1/*2* <22 g/week 22–197 g/week 198–395 g/week *ALDH2*2/*2* <22 g/week *ADH1B*1/*1* <22 g/week 22–197g/week 198–395 g/week ≥396 g/week Former drinker *ADH1B*1/*2 or *2/*2* <22 g/week 22–197g/week 198–395 g/week ≥396 g/week Former drinker	7.5 (4.7–11.8) 4.1 (2.1–8.1) 0.9 (0.5–1.7) 0.0 (not calculable) 1 5.6 (1.5–20.3) 10.4 (2.9–37.8) 8.8 (1.5–50.8) 0.8 (0.1–4.1) 5.8 (1.6–21.4) 50.5 (9.2–278) 1.4 (0.2–9.5) 4.3 (0.4–44) 4.0 (1.0–15.5) 33.3 (11.1–99.5) 38.6 (13.3–112.5) 19.6 (1.7–233) 0.2 (0.06–0.7) 1 4.1 (2.3–7.4) 7.0 (3.8–13.0) 5.7 (2.0–16.2)	Age, strong alcoholic beverage, smoking, green-yellow vegetables and fruit (drinking, *ALDH2*, *ADH1B* and ADH1C genotypes for overall)	Multivariate odds ratio for *ALDH2*2/*2* in comparison with *ALDH2*1/*1* was 7.8 (1.3–46.1); however, most men with **2/*2* genotype drank rarely or never and the risk was evaluated based on a small sample size (2 cases/43 controls). For *ADH1C* genotype, the relative risk is associated with less active *ADH1C*1/*1* versus active **1/*2* or **2/*2*. When the linkage disequilibrium between *ADH1B* and *ADH1C* was taken into consideration, the *ADH1C* genotype did not significantly affect the risk for cancer.

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Muto *et al.* (2005), Kashiwa, Japan, 1999–2001	Multiple primary squamous-cell carcinoma in both the oesophagus and head and neck	40 (37 men) from National Cancer Center Hospital East, aged 29–86 years, Japanese	163 (140 men, 23 women) with single-organ squamous-cell carcinoma of the oesophagus or head and neck from the hospital, aged 29–86 years, Japanese	Structured interview	Overall *ALDH2*	 5.5 (2.4–12.6)	Age, sex	
Wu *et al.* (2005), Kaohsiung, Taiwan, China, 2000–03	Oesophageal squamous-cell carcinoma	134 men from Kaohsiung Veterans General Hospital and Kaohsiung Medical University Hospital, aged 59±13 years, Chinese	237 hospital-based healthy men from the hospitals, aged 58±12 years; matched by age	Structured interview	Overall *ALDH2* *ADH1B* *ALDH2*1/*1* *ADH1B*1/*1* ≤1500 g/year >1500 g/year *ALDH2*1/*1* *ADH1B*1/*2* or **2/*2* 0 ≤1500 g/year >1500 g/year *ALDH2*1/*2* *ADH1B*1/*1* 0 ≤1500 g/year	 5.3 (2.5–11.2) 7.1 (2.7–18.5) versus **2/*2* 14.9 (1.9–116) 33.5 (3.5–320) 1 3.8 (0.7–21.7) 6.1 (1.5–25.3) 18.6 (2.7–129) 139 (10.1–∞)	Age, smoking, education, areca chewing, (drinking, *ALDH2* and *ADH1B* genotypes for overall)	

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Wu *et al*. (2005) (contd)					*ALDH2*1/*2 ADH1B *1/*2* or **2/*2*			
					0	2.9 (0.7–12)		
					≤1500 g/year	26.6 (6.1–118)		
					>1500 g/year	39.3 (7.1–218)		
					*ALDH2*2/*2 ADH1B*1/*2 or *2/*2*			
					0	2.2 (0.3–14.5)		
Yang *et al*. (2005), Aichi, Japan, 2001–04	Oesophageal cancer	165 (148 men; 159 squamous-cell carcinoma, 6 adenocarcinoma) from Aichi Cancer Center Hospital, aged 61±1 years; Japanese	495 hospital-based (444 men) from the hospital, matched by age and sex, aged 61±0 years, Japanese; response rate, approximately 60%	Structured questionnaire	Overall		Age, smoking, (drinking for overall)	
					ALDH2	6.4 (4.0–10.3)		
					ADH1B	0.62 (0.2–1.7) versus **2/*2*		
					*ALDH2*1/*1*			
					0 g/week	1		
					≤250 g/week	1.9 (0.4–8.4)		
					>250 g/week	4.6 (0.9–23.1) Interaction $p<0.01$		
					*ALDH2*1/*2*			
					0 g/week	1		
					≤250 g/week	9.6 (3.2–28.8)		
					>250 g/week	95.4 (28.7–317)		
Cai *et al*. (2006), Taixing City, China, 2000	Oesophageal squamous-cell carcinoma	218 (141 men) from the Taixing Tumor Registry, aged ≥20 years, Chinese; response rate, 68%	415 population-based, Chinese; matched by age, sex, village; response rate, 90%	Structured interview	*ALDH2*1/*1*	1	Age, sex, drinking, smoking, education, body mass index	Taixing City has a very high incidence rate (65/100 000) of oesophageal cancer; alcohol drinking was not significantly associated with the cancer risk; *ALDH2* genotype may modify the low-selenium intake-associated risk.
					*ALDH2*1/*2*	0.8 (0.5–1.2)		
					*ALDH2*2/*2*	1.7 (0.9–3.5)		
					*ALDH2*1/*1 or *1/*2*	1		
					*ALDH2*2/*2*	1.91 (0.96–3.80)		

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Chen *et al.* (2006), Taipei, Kaohsiung, Taiwan, China, 2000–04	Oesophageal squamous-cell carcinoma	330 men from National Taiwan University Hospital, Kaohsiung Veterans General Hospital and Kaohsiung Medical University Hospital, aged 60±12 years, Chinese	592 men from the hospitals, aged 59±11 years; matched by age	Structured interview	Overall *ALDH2*1/*1* *ALDH2*1/*2* *ALDH2*2/*2* *ADH1B*1/*1* *ADH1B*1/*2* *ADH1B*2/*2* *ALDH2*1/*1* 0 <1200 g/year ≥1200 g/year *ALDH2*1/*2* 0 <1200 g/year ≥1200 g/year *ALDH2*2/*2* 0 ≥1200 g/year *ADH1B*1/*1* 0 <1200 g/year ≥1200 g/year *ADH1B*1/*2* 0 <1200 g/year ≥1200 g/year *ADH1B*2/*2* 0 <1200 g/year ≥1200 g/year	 1 5.0 (3.1–8.0) 4.2 (1.5–11.8) 4.0 (2.1–7.5) 1.2 (0.8–1.9) 1 1 3.1 (1.3–7.5) 7.2 (3.0–17) 1.3 (0.6–3.0) 42.5 (16.9–107) 30.5 (12.0–77.6) 1.4 (0.4–4.6) 39.8 (2.4–654) 1.7 (0.4–6.6) 26.3 (9.2–74.8) 147 (41.4–525) 0.8 (0.3–1.6) 14.3 (6.2–33.0) 20 (8.5–47) 1 12.0 (5.5–26.2) 9.7 (4.4–21.3)	Age, ethnicity, smoking, education, areca chewing, *ALDH2* or *ADH1B* genotypes (drinking for overall)	The effect of *ALDH2*2/*2* was evaluated based on a small sample size of drinkers. Non-drinkers: 7 cases/40 controls; <1200 g/year: 1 case/0 control; ≥1200 g/year: 2 cases/1 control

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Hashibe *et al.* (2006), Czech Republic, Poland, Romania, Russia, Slovakia, 2000–02	Upper aerodigestive tract squamous-cell carcinoma	811 (713 men; 168 oral, 113 pharyngeal, 326 laryngeal, 176 oesophageal), from multiple centres; Romania, 142; Poland, 206; Russia, 365; Slovakia, 40; Czech Republic, 58; response rate, 90%	1083 multicentre hospital-based (831 men); Romania, 173; Poland, 209; Russia, 319; Slovakia, 84; Czech Republic, 298; matched by age, sex	Structured interview	*ADH1B* Overall Oral Pharynx Larynx Oesophagus	 2.1 (1.4–3.1) 2.0 (0.96–4.3) 1.7 (0.7–4.2) 1.8 (1.04–2.9) 5.2 (1.9–14.3)	Age, sex, country, drinking, smoking	*ALDH2* +82A>G, +348C>T and –261C>T showed linkage disequilibrium and were associated with risk for overall and oesophageal squamous-cell carcinoma.
Hashimoto *et al.* (2006), Yamaguchi, Japan, 2002–04	Head and neck cancer	192 (146 men; 98 oral, 41 pharyngeal, 47 laryngeal, 6 nasal and sinuses) from Yamaguchi University Hospital, aged 24–91 years, Japanese; response rate, 96%	192 hospital-based (146 men), aged 24–91 years, Japanese; matched by age, sex	Interview, from cases only	Cases versus controls *ALDH2* Case drinkers *ALDH2*	Not significantly different Significantly increased (p<0.009) in cases <66 years compared with cases ≥66 years	None	More cases <66 years were drinkers than cases ≥66 years.
Yokoyama *et al.* (2006a) Tokyo, Chiba, Kanagawa, Osaka, Japan 2000–04	Oesophageal squamous-cell carcinoma	52 women from Tokyo, Chiba, Kanagawa and Osaka hospitals, aged 40–79 years, Japanese; response rate, 100%	412 cancer-free women who underwent an annual medical check-up at one of two Tokyo clinics, aged 40–79 years, Japanese; response rate, 82%	Structured questionnaire	*ALDH2*1/*1* <22 g/week 22–197g/week 198–395 g/week ≥396 g/week *ALDH2*1/*2* <22 g/week 22–197 g/week 198–395 g/week ≥396 g/week	 1 0.8 (0.2–2.6) 2.0 (0.5–7.7) 3.2 (0.7–15.5) 0.5 (0.2–1.3) 2.0 (0.5–7.1) 4.7 (0.7–31) 59 (4.7–750)	Age, smoking, green-yellow vegetables and fruit, hot food and beverages	

Table 4.4 (continued)

Reference, study location, period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI)[a]	Adjustment factors	Comments
Asakage *et al*. (2007), Tokyo, Chiba, Kanagawa, Osaka, Japan 2000–03	Oral and pharyngeal squamous-cell carcinoma	96 men (43 hypo-pharyngeal, 53 oral/oropharyngeal) from Tokyo, Chiba, Kanagawa, and Osaka hospitals, aged 40–79 years, Japanese	642 cancer-free men who underwent an annual medical check-up at one of two Tokyo clinics, aged 40–79 years; Japanese; response rate, 86%;	Structured questionnaire	Moderate-to-heavy drinkers (22 g/drink, ≥9 drinks/ week) *ALDH2* *ADH1B* *ADH1C*	 3.6 (2.0–6.7) 5.6 (2.3–13.6) 3.2 (1.4–7.5)	Age, drinking, smoking, intake of green-yellow vegetables	When the linkage disequilibrium between *ADH1B* and *ADH1C* was taken into consideration, the *ADH1C* genotype did not significantly affect the risk for cancer.
	Hypopharyngeal squamous-cell carcinoma	43 men			*ALDH2* *ADH1B* *ADH1C*	10.1 (3.8–26.8) 7.2 (2.4–22.1) 2.8 (0.8–10.3)		
	Oral/oro-pharyngeal squamous-cell carcinoma	53 men			*ALDH2* *ADH1B* *ADH1C*	1.8 (0.8–3.9) 4.2 (1.4–12.6) 4.3 (1.7–11.2)		

[a] Associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1*, less active *ADH1B*1/*1* or *ADH1C*1/*1* versus active **1/*2* or **2/*2* ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CI, confidence interval; UOEH, University of Occupational and Environmental Health

Table 4.5 Cohort studies of *ALDH2* and *ADH1B* genotype-associated risk for cancer (upper aerodigestive tract)

Reference, location	Cohort description	Exposure assessment	Cancer and site	Exposure categories	No. of subjects/ squamous-cell carcinoma	Hazard ratio (95% CI)	Adjustment factors	Comments
Yokoyama *et al.* (1998b), Kanagawa, Japan	34 Japanese alcoholic men who underwent endoscopic mucosectomy for carcinoma *in situ* or mucosal squamous-cell carcinoma of the oesophagus during 1993–97; endoscopic follow-up from 6 to 48 months (mean, 22 months)	*ALDH2* genotyping	Oesophageal squamous-cell carcinoma, metachronous primary	Active *ALDH2*1/*1* Inactive *ALDH2*1/*2*	15/1 19/8	1 7.6 (0.9–61)	Not described	The log-rank test showed a significant effect of *ALDH2* genotype ($p<0.024$).
Yokoyama *et al.* (2006b), Kanagawa, Japan	808 Japanese alcoholic men confirmed cancer-free by endoscopic screening during 1993–2005; endoscopic follow-up from 1 to 148 months (median, 31 months)	*ALDH2*, *ADH1B* genotyping at baseline examination in 556 patients	Upper aerodigestive tract squamous-cell carcinoma	Active *ALDH2*1/*1* Inactive *ALDH2*1/*2* Active *ADH1B*1/*2* and **2/*2* Less-active *ADH1B*1/*1*	484/27 72/26 381/28 175/25	1 11.6 (5.7–23.3) 1 2.0 (1.02–4.0)	Age	

Table 4.5 (continued)

Reference, location	Cohort description	Exposure assessment	Cancer and site	Exposure categories	No. of subjects/ squamous-cell carcinoma	Hazard ratio (95% CI)	Adjustment factors	Comments
Yokoyama *et al.* (2006b) (contd)			Oesophageal squamous-cell carcinoma	Active *ALDH2*1/*1*	484/14	1		
				Inactive *ALDH2*1/*2*	72/19	13.0 (5.2–32.1)		
				Active *ADH1B*1/*2* and **2/*2*	381/18	1		
				Less-active *ADH1B*1/*1*	175/15	1.6 (0.7–3.9)		
			Oropharyngo-laryngeal squamous-cell carcinoma	Active *ALDH2*1/*1*	484/17	1		
				Inactive *ALDH2*1/*2*	72/13	11.7 (4.7–29.5)		
				Active *ADH1B*1/*2* and **2/*2*	381/16	1		
				Less-active *ADH1B*1/*1*	175/14	2.0 (0.8–5.0)		

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CI, confidence interval

Table 4.6 Case–control studies of *ALDH2*, *ADH1B* and *ADH1C* genotype-associated risk for cancerof the liver, colorectum and breast

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Hepatocellular carcinoma							
Shibata *et al.* (1998), Kurume, Japan, 1992–95	115 men (15 HBsAg-positive, 96 anti-HCV-positive) from Kurume University Hospital, aged 40–74 years, Japanese	115 hospital- (1 HBsAg-positive, 8 anti-HCV-positive) and 115 population-based men, aged 40–74 years, Japanese; matched by age	Self-administered questionnaire	*ALDH2* Versus hospital controls Versus community controls	**2/*2* or **1/*2* versus **1/*1* 1.1 (0.6–2.5) 0.5 (0.2–1.0)	Not described	The frequency (38%) of *ALDH2*1/*1* in the community controls was lower than that generally reported in Japan.
Yokoyama *et al.* (1998a), Kanagawa, Japan, 1987–97	18 alcoholic men (13 incident cases, 5 prevalent cases) from Kurihama National Hospital, aged 56±7 years, Japanese	487 cancer-free alcoholic men from the hospital, aged 53±8 years, Japanese	Structured interview	Alcoholics *ALDH2*	0.7 (0.1–5.6)	Age, drinking, smoking	

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Koide *et al*. (2000), Nagoya, Japan, 1994	84 (64 men; 12 HBsAg-positive, 68 anti-HCV-positive) from Nagoya City University Hospital and its affiliated hospital, aged 46–79 years, Japanese	84 population-based (0 HBsAg-positive, 6 anti-HCV-positive) from the same resident community, Japanese; matched by age, sex	Structured interview	Overall *ALDH2*	0.80 (0.5–1.4)	Age, sex	Alcoholic beverage drinking was not a significant risk factor.
Takeshita *et al*. (2000a), Hyogo, Japan, 1993–96	102 (85 men; 8 HBsAg-positive, 71 anti-HCV-positive) from 20 hospitals, aged 62±8 years (men) and 65±6 years (women), Japanese	125 hospital-based (101 men; 0 HBsAg-positive, 0 anti-HCV-positive) from the same hospitals, aged 60±12 years (men) and 63±13 years (women), Japanese; matched by age, sex	Self-administered questionnaire	Overall *ALDH2* *ADH1B*	1.1 (0.6–2.1) **1/*1* or **1/*2* versus **2/*2* 1.3 (0.7–2.0)	Age, smoking	Alcoholic beverage drinking was a significant risk factor.
Yu *et al*. (2002), Haimen, China, 1995–97	248 (207 men; 91 HBsAg-positive, 7 anti-HCV-positive) from Haimen People's Hospital, aged 25–79 years, Chinese	248 population-based (207 men; 21 HBsAg-positive, 8 anti-HCV-positive), Chinese; matched by age, sex, residence	Structured interview	Overall *ALDH2*	**2/*2* or **1/*2* versus **1/*1* 0.72 (0.5–1.2)	None	Alcoholic beverage drinking was not a significant risk factor.

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Kato *et al*. (2003), Tokyo, Japan	99 (82 men; 99 anti-HCV-positive) from Nippon Medical School, aged 42–78 years, Japanese	135 hospital-based (104 men; 0 anti-HCV-positive), aged 32–81 years, Japanese; matched by age, sex	Not described	Overall *ALDH2*	**2/*2* versus **1/*2* or **1/*1* 5.4 (2.1–14.0)	None	20% of patients had *ALDH2*2/*2*; the rate is much higher than that in the other studies (2–10%).
Munaka *et al*. (2003), Fukuoka, Japan, 1997–98	78 (61 men; 14 HBV, 54 HCV, 8 HBV+HCV) from UOEH hospital, aged 47–84 years, Japanese	138 hospital-based unmatched (94 men; 1 HBV, 10 HCV), aged 34–92 years, Japanese	Structured interview	Overall *ALDH2*	**2/*2* or **1/*2* versus **1/*1* 1.5 (0.9–2.7) 9.8 (1.6–58.6)	Age, sex Age, sex, drinking, HCV, HBV	Alcoholic beverage drinking was a significant risk factor.
Covolo *et al*. (2005), Brescia, Pordenone, Italy, 1999–2002	200 (79% men; 22 HBsAg-positive, 92 HCV RNA-positive) from 5 hospitals in northern Italy, mean age, 66.5±8 years; response rate, ≥95%	400 hospital-based (79% men; 10 HBsAg-positive, 19 HCV RNA-positive), matched by age, sex, date, hospital of admission; response rate, ≥95%	Structured interview	Overall *ADH1C*	**1/*1* versus **1/*2* or **2/*2* 0.8 (0.5–1.3)	Age, sex, area of recruitment, HCV, HBV	Alcoholic beverage drinking was a significant risk factor.

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Sakamoto *et al*. (2006), Saga, Japan, 2001–04	209 (141 men; 13 HBsAg-positive, 173 anti-HCV-positive, 6 both positive) from Saga Medical School Hospital and Saga Prefectural Hospital, aged 40–79 years, Japanese; response rate, 92%	275 hospital-based (180 men; 6 HBsAg-positive, 21 anti-HCV-positive) from Saga Medical School Hospital, aged 40–79 years, Japanese; response rate, 73% 381 hospital-based chronic liver disease (205 men; 20 HBsAg-positive, 266 anti-HCV-positive, 3 both positive) from the 2 hospitals, aged 40–79 years, Japanese; response rate, 96%	Structured interview	Light-to-moderate drinkers (<69 g ethanol/day *ALDH2* Hospital controls Chronic liver disease controls	 4.4 (1.2–15.4) 1.8 (0.8–3.7)	Age, sex, smoking, HCV, HBV	Alcoholic beverage drinking was a significant risk factor; no *ALDH2*-associated risk observed in non-drinkers or heavy drinkers; there were no significant interactions between current drinking status and *ADH1B* genotype.

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Colon cancer							
Yokoyama *et al*. (1998a), Kanagawa, Japan, 1987–97	46 alcoholic men (35 incident cases, 11 prevalent cases) from Kurihama National Hospital, aged 58±9 years, Japanese	487 cancer-free alcoholic men from the hospital, aged 53±8 years, Japanese	Structured interview	Alcoholics *ALDH2*	3.4 (1.5–7.4)	Age, drinking, smoking	
Colorectal cancer							
Murata *et al*. (1999), Chiba, Japan, 1989–95	270 (163 men; 160 colon, 110 rectum) from Chiba Cancer Center Hospital, Japanese	121 hospital-based (60 men), Japanese	Self-administered questionnaire	Male colon cancer *ALDH2*1/*1* (mL ethanol / day) 0 2.7–27 ≥27 *ALDH2*1/*2* 0 0.1–1.0 ≥1.0	 1.0 (reference) 1.3 (0.2–8.6) 1.9 (0.4–8.6) 1.0 (reference) 1.6 (0.3–7.8) 3.1 (0.7–14.0)	Age	The number of *ALDH2*2* alleles was more frequent in colon cancer cases (trend p=0.04), but not rectal cancer cases (trend p=0.21), compared with controls; trend p adjusted for sex only; odds ratios for each genotype not shown.

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Murata *et al*. (1999) (contd)				Male rectal cancer *ALDH2 *1/*1* (mL ethanol / day) 0 2.7–27 ≥27 *ALDH2 *1/*2* 0 2.7–27 ≥27	 1.0 (reference) 0.9 (0.1–5.8) 1.4 (0.4–5.1) 1.0 (reference) 0.7 (0.1–3.7) 1.3 (0.2–7.0)		

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Matsuo *et al.* (2002), Aichi, Japan, 1999	142 (83 men; 72 colon, 70 rectum) from Aichi Cancer Center Hospital, Japanese	241 (118 men), from the hospital, Japanese	Self-administered questionnaire	Overall *ALDH2* Men **1/*1* **1/*2* **2/*2* Women **1/*1* **1/*2* **2/*2* Alcohol drinking *ALDH2 *1/*1* Low Moderate High *ALDH2 *1/*2* Low Moderate High *ALDH2*2/*2* Low Moderate High	 1.0 (reference) 0.7 (0.4–1.3) 0.4 (0.1–1.5) 1.0 (reference) 1.1 (0.6–2.2) 0.6 (0.2–2.5) 1.0 (reference) 1.2 (0.5-2.6) 1.9 (0.8-4.8) Trend p=0.14 1.0 (ref) 0.8 (0.3–2.0) 3.6 (1.0–13.0) Trend p=0.16 1.0 (reference) 24.5 (0.8–787) Not calculated Trend p=0.07	Age, smoking in the overall analysis; age, sex in the stratified analysis	Alcohol category: low (less than once), moderate (≥1 per week with <50 mL ethanol), high (≥1 per week with ≥50 mL ethanol); increased risk associated with alcohol in *ALDH2*1/*2* was seen for rectal cancer (trend p=0.01), not for colon cancer (trend p=0.44).

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Landi *et al.* (2005), Barcelona, Spain	377 from a hospital	326 non-cancer patients at the same hospital	None	Overall *ADH1B* **1/*1* **1/*2* **2/*2*	 1.0 (reference) 1.0 (0.7–1.6) 0.6 (0.1–3.5)	Age, sex	Alcohol beverage intake not ascertained
Otani *et al.* (2005), Nagano, Japan; 1998–2002	107 (66 men) from 4 hospitals in Nagano Prefecture	224 healthy (141 men) from among those receiving medical check-up; matched for hospital, sex, age (±3 years), residence area	Self-administered questionnaire	Overall *ALDH2* **1/*1* **1/*2* **2/*2*	 1.0 (reference) 1.1 (0.7–1.9) 1.2 (0.5–2.9)	Age, sex, residence, hospital	No stratification with alcohol intake
Matsuo *et al.* (2006a), Aichi, Japan, 2001–04	257 (162 men; 123 colon, 131 rectum, 3 both) from Aichi Cancer Center Hospital, aged 59±10 years, Japanese	771 hospital-based (486 men), aged 59±10 years, Japanese; matched by age, sex	Self-administered questionnaire	Overall *ALDH2* **1/*1* **1/*2* **2/*2* *ADH1B* **1/*1* **1/*2* **2/*2*	 1.0 (reference) 1.0 (0.7–1.4) 1.0 (0.5–1.8) 1.9 (1.1–3.5) 1.4 (1.0–1.8) 1.0 (reference)	Age, sex, drinking, smoking, body mass index, family history, estrogen use; conditions with potential use of NSAIDs	A strong interaction between *ALDH2* and *ADH1B* was noted ($p<0.001$); the association with alcohol was examined with the composite genotype stratified (see test).

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Breast cancer							
Freudenheim *et al*. (1999), western New York, USA, 1986–91	315 women (134 premenopausal, 181 postmenopausal) from major hospitals in Erie and Niagara counties, aged 40–85 years, Caucasian; 66% of eligible premenopausal cases, 54 % of eligible postmenopausal cases	356 population-based (126 premenopausal, 230 postmenopausal), aged 40–85 years, Caucasian; 62% of eligible premenopausal cases, 44 % of eligible postmenopausal cases	Structured interview	Premenopausal *ADH1C*1/*1* Lower Higher *ADH1C*1/*2* and **2/*2* Lower Higher Postmenopausal *ADH1C*1/*1* Lower Higher *ADH1C*1/*2* and **2/*2* Lower Higher	 1.0 (0.4–2.5) 3.6 (1.5–8.8) Interaction p=0.16 1 0.8 (0.4–1.7) 0.9 (0.5–1.6) 1.2 (1.1–2.2) 1 0.8 (0.5–1.4)	Age, education, body mass index, parity, age at first birth, age at menarche, fruit and vegetable intake, duration of lactation, benign breast disease, age at menopause	The cut-off between lower and higher alcoholic beverage intake was 6.5 and 4.5 drinks per month on average over the past 20 years for the pre- and postmetnopausal women, respectively.

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Hines *et al*. (2000), 11 states, USA, 1989–94	465 women of 32 826 cohort members in 11 states, 85% Caucasian	621 population-based from the cohort, Caucasian; 85% matched by birth years, menopausal status, hormone use	Self-administered questionnaire	*ADH1C*1/*1* 0 g ethanol/day ≤10 g/day >10 g/day *ADH1C*1/*2* 0 g/day ≤10 g/day >100 g/day *ADH1*2/*2* 0 g/day ≤10 g/day >100 g/day	 1 0.8 (0.5–1.3) 0.8 (0.4–1.5) Interaction p=0.15 0.7 (0.4–1.2) 1.1 (0.7–1.8) 0.8 (0.4–1.4) 0.6 (0.3–1.2) 0.6 (0.3–1.2) 1.1 (0.5–2.4)	Age of birth, drinking, body mass index, parity, age at menarche, family history, benign breast disease	
Choi *et al*. (2003), Seoul, Republic of Korea, 1995–2001	346 women (226 premenopausal, 120 postmenopausal) from 3 hospitals in Seoul, aged 47±10 years, Korean	377 hospital-based women (209 premenopausal, 168 postmenopausal), aged 47±14 years, Korean	Structured interview	Overall *ALDH2*	 0.8 (0.6–1.2)	Age, family history	

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B, ADH1C* genotypes	Adjustment factors	Comments
Coutelle *et al*. (2004), Heidelberg, Germany	117 women from the University Hospital of Heidelberg, aged 53±12 years, Caucasian	111 alcoholics (74 cirrhosis, 22 pancreatitis, 15 heavy drinkers), aged 57±11 years, Caucasian; matched by age	Interview	Overall *ADH1C* **1/*1*, **1/*2* or **2/*2*	 1.8 (1.4–2.3) 1	Not described	Alcohol intake: cases, 17±22 g/day; alcoholic controls, 110±89 g/day
Lilla *et al*. (2005), southern Germany, 1992–95	613 women aged ≤50 years, from 38 hospitals; 61% of eligible cases, aged 42±6 years	1082 population-based; 48% of eligible controls, aged 43±6 years	Self-administered questionnaire	*ADH1B*1/*1* 0 g ethanol/day ≥12 g/day *ADH1B*1/*2* and **2/*2* 0 g/day ≥12 g /day	 1 1.1 (0.8–1.6) 1 0.3 (0.1–1.0) Interaction p=0.05	Age, education, smoking, family history, menopausal status, breast-feeding	Interactions between other drinking categories and *ADH1B* genotype not significant

Table 4.6 (continued)

Reference, study location, period	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) associated with inactive heterozygous *ALDH2*1/*2* versus active **1/*1* and *ADH1B*, *ADH1C* genotypes	Adjustment factors	Comments
Terry *et al.* (2006), New York, USA, 1996–97	1047 women, from the Long Island Breast Cancer Study Project; 70% of eligible cases; English speakers	1101 population-based; 70.7% of eligible controls; English speakers	Structured interview	Lifetime intake *ADH1C*1/*1* 0 g ethanol/day 15–30 g/day ≥30 g/day *ADH1C *1/*2* 0 g/day 15–30 g/day ≥30 g/day *ADH1C *2/*2* 0 g/day 15–30 g/day ≥30 g/day	 1 2.0 (1.1–3.5) 0.8 (0.4–1.7) Interaction p=0.20 1 1.5 (0.9–2.4) 0.8 (0.4–1.5) 1 1.3 (0.5–3.5) 0.9 (0.2–3.4)	Age, education, race, caloric intake, smoking, body mass index, history of benign breast disease, parity, age at first birth, age at menarche, menopausal and lactation status	The association for *ADH1C*1/*1* carriers who drank 15–30 g/day was more pronounced among premenopausal women (odds ratio, 2.9; 95% CI, 1.2–7.1) versus postmenopausal women (odds ratio, 1.8; 95% CI, 0.9–3.8).

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CI, confidence interval; HbsAg, hepatits B virus surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; NSAIDS, non-steroidal anti-inflammatory drugs

the mechanisms by which the functional polymorphism affects cancer susceptibility has not been fully explained. The evidence of a relationship between the *ADH1B* genotype and cancer in other organs is inconclusive because of the small number of studies.]

(ii) *ADH1C*

ADH1C (previously called *ADH3*) gene polymorphism is a major polymorphism among Caucasians. The homodimer encoded by the *ADH1C*1* allele catalyses the production of acetaldehyde from ethanol at a rate 2.5 times faster than the homodimer encoded by the *ADH1C*2* allele (reviewed in Bosron & Li, 1986). In a follow-up study of Australian twins the *ADH1C* genotype showed a considerably weaker effect on drinking behaviour than did the *ADH1B* genotype; however, among *ADH1B*1/*1* men, *ADH1C*1/*1* carriers were less likely to become alcoholics (Whitfield *et al.*, 1998). A meta-analysis of 11 case–control studies of alcoholics failed to show such an *ADH1C*-associated risk in Caucasians (Zintzaras *et al.*, 2006). Two alcohol-challenge tests reported inverse results: higher salivary concentrations of acetaldehyde were found in healthy Caucasians with *ADH1C*1/*1* than in those with *ADH1C*2* (Visapää *et al.*, 2004) and lower breath concentrations of acetaldehyde were measured in *ADH1C*1/*1* carriers than in *ADH1C*2* carriers among Japanese cancer patients with an inactive *ALDH2*2* allele (Muto *et al.*, 2002).

Fourteen case–control studies in populations exclusively or mainly composed of Caucasians have investigated associations between *ADH1C* genotype and upper aerodigestive tract cancer, but showed no consistent pattern of association (Table 4.7). A higher *ADH1C*1/*1*-associated risk was shown in five studies: for laryngeal cancer in a small population of alcoholics (Coutelle *et al.*, 1997), for oral and pharyngeal squamous-cell carcinoma in heavy alcoholic beverage drinkers (Harty *et al.*, 1997), for upper aerodigestive tract cancer in comparison with control patients with alcoholic cirrhosis, alcoholic pancreatitis or alcoholism (Visapää *et al.*, 2004; Homann *et al.*, 2006) and for upper aerodigestive tract squamous-cell carcinoma in a large central-European population (811 cases, 1083 controls; Hashibe *et al.*, 2006). However, the same central-European study (Hashibe *et al.*, 2006) yielded no association when the linkage disequilibrium between *ADH1B*2* and *ADH1C*1* was taken into consideration. Negative results were reported in six other studies (Bouchardy *et al.*, 2000; Olshan *et al.*, 2001; Sturgis *et al.*, 2001; Zavras *et al.*, 2002; Risch *et al.*, 2003; Wang *et al.*, 2005a). A pooled analysis of data from seven case–control studies with a total of 1325 cases and 1760 controls confirmed the negative results (Brennan *et al.*, 2004), but three others reported an interaction between *ADH1C*2 /*2* and alcoholic beverage drinking (Schwartz *et al.*, 2001; Nishimoto *et al.*, 2004; Peters *et al.*, 2005). The direction and magnitude of interaction may have differed because of differences in alcohol consumption, ethnicity and linkage disequilibrium between *ADH1C* and *ADH1B* among the study populations.

East Asian case–control studies have consistently demonstrated an *ADH1C*2*-associated risk for alcoholism (Zintzaras *et al.*, 2006). Two Japanese case–control studies reported that the *ADH1C*2* allele increases the risk for oral/oropharyngeal cancer,

Table 4.7 Case–control studies of *ADH1C*-genotype-associated risk for cancer of the upper aerodigestive tract (non-Asians)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Coutelle *et al*. (1997), Bordeaux, France	Oropharyngeal and laryngeal cancer	39 alcoholic cancer patients (21 oropharynx, 18 larynx), mean age, 54 yrs, Caucasian.	37 alcoholic men from an alcoholism clinic, mean age, 42 years, Caucasian.	Not described	*ADH1C *1/*1* vs **1/*2+*2/*2* Overall Oropharyngeal Laryngeal	 3.6 (0.7–10.0) 2.6 (0.7–10.0) 6.1 (1.3–28.6)	Age	All subjects consumed more than 100 g ethanol/ day for more than 10 years.
Harty *et al*. (1997), Puerto Rico, 1992–95	Oral and pharyngeal squamous-cell carcinoma	137 (123 men), from the Puerto Rico Cancer Registry, aged 21–79 years, 48 % response rate, white 91, black 15, mestizo 18, other 13	146 population-based controls (112 men), 57% response rate, white 102, black, 10, mestizo 24, other 10	Structured interview	Heavy drinkers *ADHIC* **1/*1* **1/*2 + *2/*2* Risk elevation per additional drink/week **1/*1* **1/*2 +*2/*2*	 5.3 (1.0–28.8) 1 3.6% (1.9–5.4%) 2.0% (0.9–3.0%)	Age, sex, tobacco, fruit and vegetable consumption	Heavy drinkers ≥57 drinks/ week: 46% cases, 9% controls
Bouchardy *et al*. (2000), France, 1988–92	Oral, pharyngeal, and laryngeal squamous-cell carcinoma	121 (113 men; 67 oral, 50 pharyngeal, 4 unspecified), aged 54±10 years, 129 (127 men, 2 women; 129 laryngeal), aged 55±9 years, Caucasian	172 hospital-based controls (163 men), regular smokers, matched by age, sex and hospital, aged 55±11 years	Structured interview	*ADH1C* Oral/ pharynx **1/*1* **1/*2* **2/*2* Larynx **1/*1* **1/*2* **2/*2*	 1.1 (0.6–2.2) 0.7 (0.4–1.4) 1 0.7 (0.4–1.4) 1.0 (0.5–1.8) 1	Age, sex, drinking, smoking	Heavy drinkers >80 g/day: 59% oral/ pharyngeal cases, 60% laryngeal cases, 37% controls

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Olshan *et al*. (2001), North Carolina, USA, 1994–97	Head and neck squamous-cell carcinoma	182 (76% men; 93 oral, 37 pharyngeal, 52 laryngeal) from University of North Carolina Hospital, aged >17 years, 88% response rate, 62% white, 38% black	202 hospital-based controls (56% men), matched by age and sex, 86% response rate, 86% white, 14% black	Structured interview	*ADH1C* **1/*1* **1/*2* **2/*2*	 0.9 (0.4–1.9) 0.8 (0.4–1.7) 1	Age, sex	Heavy drinkers ≥60 drinks/ week: 23% cases, 3% controls. No interaction between alcohol drinking and *ADH1C* genotype
Sturgis *et al*. (2001), Houston, USA, 1995–2000	Oral and pharyngeal squamous-cell carcinoma	229 (145 men), from Anderson Cancer Center, 90% response rate, non-Hispanic white	575 hospital-based controls (340 men), from a multispecialty managed-care institute, matched by age, sex and smoking, 73% response rate, non-Hispanic white	Questionnaire	*ADH1C* **1/*1* **1/*2* **2/*2*	 1 1.0 (0.7–1.4) 1.2 (0.8–1.9)	Age, sex, drinking, smoking	

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Schwartz *et al.* (2001), Washington, USA, 1985–89, 1990–95	Oral squamous-cell carcinoma	333 (237 men; 141 tongue, 76 tonsils/ oropharynx, 50 oral floor, 16 gum, 13 soft palate, 37 miscellaneous), from residents of the counties, aged 18–65 years, 54–63 % response rate, white 312, black 12, other 9	541 population-based controls (387 men), from residents of the counties, aged 18–65 years, 61–63% response rate, white 511, black 14, other 16	Structured interview	*ADHIC* **1/*1* **1/*2* **2/*2* Risk elevation per additional drink/week **1/*1* **1/*2* **2/*2*	 1.0 (0.7–1.5) 1.3 (1.0–1.2) 1 1.2% (0.0–2.4%) 2.5% (1.5–2.6%) 5.3% (2.1–8.5%)	Age, sex, race Age, sex, race, smoking	Heavy drinkers ≥43 drinks/ week: 17% cases, 4% controls
Zavras *et al.* (2002), Athens, Greece, 1995–98	Oral SCC	93 from 3 hospitals in Athens, Caucasian	99 hospital-based controls, matched by age and sex, Caucasian	Structured interview	Overall *ADH1C* **1/*1* **1/*2* **2/*2*	 1 0.8 (0.4–1.6) 0.9 (0.3–2.5)	Sex, drinking, smoking	
Risch *et al.* (2003), Southwest Germany, 1998–2000	Laryngeal squamous-cell carcinoma	245 (226 men) from the Rhein-Neckar Larynx Case–Control Study, aged 38–80 years, Caucasian	251 population-based controls (232 men), matched by age and sex, aged 38–80 years, Caucasian	Structured interview	*ADHIC* **1/*1* **1/*2*+**2/*2*	 1.1 (0.7-1.6) 1	Drinking, smoking	Heavy drinkers >75 g/day: 35% cases, 17% controls

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Nishimoto *et al*. (2004), São Paulo, Brazil, 1995–2001	Oral, pharyngeal, and laryngeal squamous-cell carcinoma	141 (110 men; 63 oral, 49 pharyngeal, 29 laryngeal) from Hospital do Câncer A.C. Camargo, aged 17–90 years, white 119, non-white 22	134 hospital-based unmatched controls (91 men), aged 22–90 years, white 110, non-white 24	Structured interview	*ADH1C* Lifetime alcohol intake <100 kg **1/*1+*1/*2 *2/*2* ≥100 kg **1/*1+*1/*2 *2/*2*	 1 3.8 (1.5–9.7) 1 0.52 (0.2–1.2)	Age, sex, family history	Heavy drinkers ≥100 kg: cases 74%, controls 28%. Opposite ADH1C effects between those with lifetime alcohol intake <100 kg and ≥100 kg
Visapää *et al*. (2004), Mannheim, Heidelberg, Germany	Upper aerodigestive tract cancer	107 (89 men; 16 oral, 8 oropharyngeal, 22 hypo-pharyngeal, 41 laryngeal, 20 oesophageal), from ENT Hospital Mannheim, aged 59±11 yrs, 99 smokers, Caucasian	103 hospital-based controls (67 men; 39 alcoholic cirrhosis, 38 alcoholic pancreatitis, 26 alcoholics), from Salem Medical Centre, matched by age, aged 58±9 yrs, 95 smokers, Caucasian	Structured interview	*ADH1C* **1* allele	1.7 (1.1–2.6) *vs *2* allele	Age, sex, drinking, smoking	Heavy drinkers >80 g/day: 53% cases, 100% controls; >20 g/day: 100% cases, 100% controls

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Wang *et al.* (2005a), Iowa, USA, 1994–97, 2000–02	Head and neck squamous-cell carcinoma	348 (226 men; 223 oral, 125 oropharyngeal), from the University of Iowa Hospitals & Clinics and the Iowa City Veterans Affairs Medical Center; 64% >55 yrs; 87% response rate, white 333, black 15	330 hospital-based controls (194 men), from the Iowa hospitals; 62% >55 yrs; 92% response rate, white 314, black 16	Self-administered questionnaire	*ADH1C* **1/*1* **1/*2* **2/*2*	 0.7 (0.4–1.1) 0.8 (0.5–1.2) 1	Age, drinking, smoking	Drinkers >21 drinks/ week: 41% cases, 17% controls
Peters *et al.* (2005), The greater Boston area, USA, 1999–2003	Head and neck squamous-cell carcinoma	521 (375 men; 256 oral, 149 pharyngeal, 106 laryngeal), from 9 hospitals, aged >17 years, mean age 60 yrs, 71% response rate, Caucasian 446, black 23, other 50	599 population-based controls (430 men), matched by age, sex, and town, aged >17 years, mean age 61 yrs, 41% response rate, Caucasian 540, black 21, other 37	Self-administered questionnaire	*ADH1C* **1/*1+*1/*2* Non-drinkers Light drinkers Heavy drinkers (>30 drinks/ wk) **2/*2* Non-drinkers Light drinkers Heavy drinkers (>30 drinks/ wk)	 1 0.9 (0.6–1.3) 2.3 (1.4-3.8) 0.8 (0.4–1.8) 0.9 (0.6–1.6) 7.1 (2.3–22) Interaction (p=0.05)	Age, sex, race, smoking	Heavy drinkers >30 drinks/ week: 27% cases, 9% controls

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Hashibe *et al.* (2006), Romania, Poland, Russia, Slovakia, Czech Republic, 2000–02	Upper aerodigestive tract squamous-cell carcinoma	811 (713 men; 168 oral, 113 pharyngeal, 326 laryngeal, 176 oesophageal), from multiple centres, response rate 90%; Romania 142, Poland 206, Russia 365, Slovakia 40, Czech Republic 58; 80% current smokers	1083 multi-centre hospital-based controls (831 men), matched by age and sex, Romania 173, Poland 209, Russia 319, Slovakia 84, Czech Republic 298; 40% current smokers	Structured interview	*ADH1C I350V *1/*1* (Val/Val) *ADH1C R272Q *1/*1* (Gln/Gln) *ADH1C*1* (350 Val) + *ADH1C*1* (272 Gln) + *ADH1B*1* (Arg)	1.4 (1.01–1.9) *vs *2/*2* (Ile/Ile) 1.5 (1.1–2.1) *vs *2/*2* (Arg/Arg) 1.1 (0.97–1.3) *vs* the combined slow haplotypes ADHC*1 (350 Ile) + ADHC*2 (272 Arg) + ADHB*1 (Arg)	Age, sex, country, drinking, smoking	Daily drinkers: 17% cases, 13% controls. *ADH1B* and *ADH1C* showed linkage disequilibrium.

Table 4.7 (continued)

Reference, study location, and period	Cancer site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	Relative risk (95% CI) by *ADH1C* genotype (*1, fast V_{max}; *2, slow V_{max})	Adjustment factors	Comments
Homann *et al*. (2006), Lübeck, Erlangen-Nürnberg, Freiburg, Regenburg, Heidelberg, Germany, 1999–2003	Upper aerodigestive tract cancer, hepatocellular carcinoma	123 oesophageal cancer (100 men; 85 squamous-cell carcinoma, 38 adeno-carcinoma), age 63±10 years, 86 head and neck cancer (73 men; 23 oral, 26 pharyngeal, 37 laryngeal), age 57±9 years, 86 alcohol-associated hepatocellular carcinoma (79 men), age 66±8 years, Caucasian	525 hospital-based controls (387 men): 217 alcoholic cirrhosis, age 57±12 years; 117 alcoholic pancreatitis, age 49±11 years, 17 cirrhosis + pancreatitis, age 53±12 years; 174 heavy drinkers, age 53±12 years, Caucasian	Interview	ADH1C 1*1 Head and neck Oesophagus Alcohol-associated hepatocellular carcinoma	 2.2 (1.1–4.4) *vs* 1*2 +2*2 2.9 (1.8–4.7) *vs* 1*2 + 2*2 3.6 (1.3–9.5) *vs* 1*2 + 2*2	Age, sex, smoking	All subjects consumed more than 40 g ethanol/day for more than 10 years.

ADH, alcohol dehydrogenase; CI, confidence interval; V_{max}, maximum velocity: activity of the enzyme encoded by the gene; *vs*, versus

hypopharyngeal cancer (Asakage *et al.*, 2007) and oesophageal cancer (Yokoyama *et al.*, 2002b) (Table 4.4). However, when the linkage disequilibrium between *ADH1B* and *ADH1C* was taken into consideration, no relationship was found between *ADH1C* genotype and cancer risk or between *ADH1C* genotype and alcoholism (Chen *et al.*, 1999). Haplotype analyses revealed that the apparent effect of the *ADH1C*2* allele reflects its linkage with the *ADH1B*1* allele, which has a true effect on the risk for cancer as well as on the risk for alcoholism. [The Working Group noted that the evidence of a contribution of the *ADH1C* polymorphism to the development of cancer in the upper aerodigestive tract is inconclusive.]

Two European case–control studies investigated associations between *ADH1C* genotype and hepatocellular carcinoma. One reported no association (Covolo *et al.*, 2005; Table 4.6), and the other found a positive association between *ADH1C*1/*1* and the risk for alcohol-associated hepatocellular carcinoma in comparison with control patients with alcoholic cirrhosis, alcoholic pancreatitis, or alcoholism (Homann *et al.*, 2006; Table 4.7). [The Working Group noted that the evidence of a relationship between *ADH1C* genotype and hepatocellular carcinoma is inconclusive because of the small number of studies.]

Four case–control studies conducted in Germany and the USA investigated the relationship between *ADH1C* genotype and the risk for breast cancer (Table 4.6). Three of them addressed an effect of the combination of *ADH1C* genotype and alcoholic beverage intake on the risk for breast cancer. Freudenheim *et al.* (1999) showed an increased risk associated with higher lifetime alcoholic beverage intake for AD*H1C*1/*1* carriers *vs ADH1C*1/*2* and *ADH1C*2/*2* carriers in both pre- and postmenopausal women, the increase being more evident in premenopausal women. Terry *et al.* (2006) reported an increased risk for breast cancer with moderate lifetime alcoholic beverage intake (15–30 g/day), but not with high intake (≥30 g/day), in women with *ADH1C*1/*1* only, and the association was more pronounced among premenopausal women. Such an interaction was not observed for any categories of current alcoholic beverage intake. There was no increase in the risk for any combination of *ADH1C* genotypes and alcoholic beverage intake in the third study (Hines *et al.*, 2000). A fourth study used patients with alcoholic cirrhosis, alcoholic pancreatitis or alcoholism as controls and showed an increased risk for *ADH1C*1/*1* compared with *ADH1C*1/*2* or *ADH1C**2/*2 (Coutelle *et al.*, 2004). [The Working Group noted that the evidence of a relationship between *ADH1C* genotype and breast cancer is inconclusive because of the small number of studies, but a few reports suggested an increased risk associated with moderate lifetime alcoholic beverage intake for the *ADH1C*1/*1* genotype in premenopausal women.]

(iii) *CYP2E1*

The enzyme CYP2E1 is induced by chronic alcoholic beverage consumption and plays a role in ethanol oxidation and the metabolic activation of many carcinogens, including *N*-nitrosamines, benzene and aniline. *CYP2E1* has various polymorphisms, and the *Pst*1- and *Rsa*1-cleavage site polymorphism (*c1/c2*) in the 5′-transcriptional

region has been the most intensively investigated. However, its functional consequence has been a matter of controversy. Early studies showed increased CYP2E1 expression and activity associated with the *c2* allele (Hayashi *et al.*, 1991; Tsutsumi *et al.*, 1994), but this finding has not been confirmed in other studies (Carrière *et al.*, 1996; Kim *et al.*, 1996; Powell *et al.*, 1998; Kato *et al.*, 2003), and contrary results have been reported (Huang *et al.*, 2003). A meta-analysis of case–control studies showed no association between the *CYP2E1* genotype and risk for either alcoholism or alcoholic liver disease (Zintzaras *et al.*, 2006). The results for cancer were inconsistent. Although two case–control studies showed that the *c1* allele increased the risk for oesophageal cancer (Tan *et al.*, 2000; Lu *et al.*, 2005), negative results were reported in eight other case–control studies (Lucas *et al.*, 1996; Hori *et al.*, 1997; Morita *et al.*, 1997; Tanabe *et al.*, 1999; Chao *et al.*, 2000; Gao *et al.*, 2002; Li *et al.*, 2005a; Yang *et al.*, 2005) and a *c2* allele-associated risk was found in yet another study (Lin *et al.*, 1998). A *c2* allele-associated risk for oropharyngolaryngeal cancer was reported in four case–control studies (Hung *et al.*, 1997; Bouchardy *et al.*, 2000; Gattás *et al.*, 2006; Sugimura *et al.*, 2006), and no association was observed in four (Lucas *et al.*, 1996; González *et al.*, 1998; Matthias *et al.*, 1998; Katoh *et al.*, 1999). A *c2* allele-associated risk for hepatocellular carcinoma was reported in three case–control studies (Ladero *et al.*, 1996; Koide *et al.*, 2000; Munaka *et al.*, 2003) and no increased risk in four others (Lee *et al.*, 1997; Wong *et al.*, 2000; Yu *et al.*, 2002; Kato *et al.*, 2003), a *c1/c1* genotype-associated risk was observed in another (Yu *et al.*, 1995). [The Working Group noted that the evidence of a contribution of the *CYP2E1* polymorphism to the development of cancer is inconclusive.]

(iv) *ALDH2*

The variant allele *2 that encodes an inactive subunit of ALDH2 is dominant and highly prevalent among East Asians (28–45%; Goedde *et al.*, 1992), but is not found in most other populations. The inactivity of ALDH2 inhibits persons from drinking heavily by causing acetaldehydaemia and alcoholic flushing responses. Most homozygotes for inactive *ALDH2*2/*2* are non-drinkers or occasional drinkers, but substantial percentages of East Asians who are habitual drinkers, including alcoholics, are heterozygous for inactive *ALDH2*1/*2* (Table 4.8).

Cancers of the upper aerodigestive tract

All case–control studies that involved 13 independent Japanese and Taiwanese (Chinese) alcoholic beverage drinking populations have shown that heterozygosity for inactive *ALDH2* is a strong risk factor for oesophageal cancer, mainly squamous-cell carcinoma (odds ratios, 4.4–16.4; reviewed in Yokoyama & Omori, 2003; see Wu *et al.*, 2005; Yang *et al.*, 2005; Chen *et al.*, 2006; Yokoyama *et al.*, 2006a; Table 4.4). A case–control study conducted in a Thai population, in which only 18% of the controls had inactive *ALDH2*, showed a marginally significant positive association (odds ratio, 1.6; Boonyaphiphat *et al.*, 2002). However, a case–control study conducted in Taixing City, China, where the incidence rate of oesophageal cancer is extremely high (65/100

Table 4.8 Relationship between *ALDH2* genotype and alcohol consumption in Japanese men

Alcoholic beverage consumption	*ALDH2* genotype		
	Homozygous active **1/*1* (*n*=341)	**Heterozygous inactive **1/*2* (*n*=250)**	**Homozygous inactive **2/*2* (*n*=43)**
Never or <22 g/week	6.2%	32.0%	95.3%
22–197 g/week	28.2%	41.2%	4.7%
198–395 g/week	39.6%	14.0%	0%
≥396 g/week	22.9%	10.8%	0%
Former drinkers	3.2%	2.0%	0%

From Yokoyama *et al.* (2002b) ALDH, aldehyde dehydrogenase

000 population), did not show a significant association between the risk for this type of cancer and inactive heterozygous *ALDH2* or with alcoholic beverage drinking (Cai *et al*., 2006). This study reported a marginally significant increased risk in inactive *ALDH2* homozygotes (odds ratio, 1.9) and suggested that inactive homozygous *ALDH2* may modify the cancer susceptibility associated with low selenium intake, an important risk factor in this high-risk population.

ALDH2-related susceptibility to oesophageal squamous-cell carcinoma in Japanese and Taiwanese (Chinese) may include light-to-moderate as well as heavy alcoholic beverage drinkers (Yokoyama *et al*., 2002b; Lewis & Smith, 2005; Wu *et al*., 2005; Yang *et al*., 2005; Chen *et al*., 2006) and female drinkers (Yokoyama *et al*., 2006a). Two prospective studies of Japanese alcoholics showed an increased risk for oesophageal squamous-cell carcinoma in heterozygotes for inactive *ALDH2* (relative hazards, 7.6 and 13.0; Yokoyama *et al*., 1998b, 2006b; Table 4.5). [The Working Group noted that the available genetic epidemiological data provide ample evidence of a strong contribution of the heterozygous *ALDH2* genotype to the development of alcohol-related cancer in the oesophagus.]

Inactive *ALDH2* has consistently been reported to be a strong risk factor for synchronous and metachronous multiple cancers in the oesophagus and oropharyngolarynx, both in Japanese alcoholics and in the general population (odds ratio, 3.4–7.4; reviewed in Yokoyama & Omori, 2003; Muto *et al*., 2005; Table 4.4). Oesophageal dysplasia is also associated with inactive heterozygous *ALDH2*, which serves as a predictor of squamous-cell carcinoma in the oesophagus and oropharyngolarynx in Japanese alcoholics (Yokoyama *et al*., 2006b); the presence of multiple areas of oesophageal dysplasia increases the risk for multiple cancers in Japanese patients with squamous-cell carcinoma of the oesophagus and oropharyngolarynx (Muto *et al*., 2002, 2005).

Other Japanese case–control studies of the *ALDH2*-associated risk for cancer of the oropharyngolarynx have reported different patterns of association according to

anatomical site and drinking habit. A study of oral cancer in which alcoholic beverage consumption was not a risk factor showed that the *ALDH2* genotype had no effect (Katoh *et al.*, 1999), but another study of oral cancer, in which alcoholic beverage consumption was a risk factor, reported a relatively weak but significantly increased risk (odds ratio, 2.9) associated with inactive heterozygous *ALDH2* (Nomura *et al.*, 2000). A case–control study of head and neck cancer, which lacked information on anatomical subsites, showed no difference in *ALDH2* genotype between cases and controls (Hashimoto *et al.*, 2006). However, the study also lacked information on the drinking status of the controls, and analysis of the association with *ALDH2* without consideration of drinking status is misleading. More cases <66 years of age were alcoholic beverage drinkers than those ≥66 years of age, and more drinking cases <66 years of age were heterozygotes for inactive *ALDH2* than drinking cases ≥66 years of age, which suggests an interaction between *ALDH2* and alcoholic beverage drinking in cases <66 years of age. A more sophisticated case–control study of oral and pharyngeal cancer showed that inactive heterozygous *ALDH2* is a strong risk factor for squamous-cell carcinoma in the hypopharynx (odds ratio, 10.1) among moderate-to-heavy drinking men, but not for squamous-cell carcinoma in the oral cavity and oropharynx (Asakage *et al.*, 2007). Although the number of cases size was small, inactive heterozygous *ALDH2* strongly increased the risk for cancer among alcoholic men in both the oral cavity/oropharynx (odds ratio, 20.8) and hypopharynx/epilarynx (odds ratio, 28.9; Yokoyama *et al.*, 2001). A prospective study of cancer-free Japanese alcoholic men showed a hazard ratio of 11.7 for oropharyngolaryngeal squamous-cell carcinoma in inactive *ALDH2* heterozygotes (Yokoyama *et al.*, 2006b; Table 4.5). [The Working Group noted that, while it is often difficult to differentiate clearly between exact locations of tumours in the oropharyngolaryngeal area based on the available published data, there is strong evidence for a contribution of heterozygous *ALDH2* genotype to the development of alcohol-related cancer in the oropharyngolarynx as a whole, and especially in the hypopharynx. However, the Group noted that epidemiological studies provide suggestive but inconclusive evidence of an association of the heterozygous *ALDH2* genotype with alcohol-related cancers in the individual oropharyngolaryngeal subsites of the oral cavity, oropharynx and larynx.]

Liver cancer

One Chinese and seven Japanese case–control studies of *ALDH2*-associated risk for hepatocellular carcinoma yielded conflicting results (Table 4.6). Most of the cases of hepatocellular carcinoma had HCV or HBV infection. Four of the Japanese studies and the Chinese study did not show an increased risk (Shibata *et al.*, 1998; Yokoyama *et al.*, 1998a; Koide *et al.*, 2000; Takeshita *et al.*, 2000a; Yu *et al.*, 2002). However, except for a study of Japanese alcoholics, all the null results were based on analyses that did not consider drinking status. One of the studies reported that the heterozygosity or homozygosity for inactive *ALDH2* was associated with a high risk for hepatocellular carcinoma by multiple regression analysis (odds ratio, 9.8; Munaka *et al.*, 2003); another study reported an interaction between inactive heterozygous *ALDH2* and

light-to-moderate alcoholic beverage drinking when using hospital controls, but not when using other controls with chronic liver disease, and that no interaction between *ALDH2* and heavy alcoholic beverage drinking was observed (Sakamoto *et al.*, 2006). A further study reported that inactive homozygous *ALDH2*2/*2* genotype was associated with an increased risk for HCV antibody-positive hepatocellular carcinoma (odds ratio, 5.4 versus other genotypes; Kato *et al.*, 2003). However, the percentage of hepatocellular carcinoma patients with the *ALDH2*2/*2* genotype in that study (20%) was much higher than that in the other studies (2–10%). Very few Japanese heavy drinkers who had hepatocellular carcinoma with negative markers for viral hepatitis were heterozygous for inactive *ALDH2* (0–12.5%; Ohhira *et al.*, 1996; Yamagishi *et al.*, 2004). [The Working Group noted that available epidemiological studies provide suggestive but inconclusive evidence of an association between heterozygous *ALDH2* genotype and hepatocellular carcinoma.]

Colorectal cancer

Five Japanese case–control studies investigated the association between *ALDH2* genotype and colorectal cancer (Table 4.6). A small study in alcoholics reported an increased risk for colon cancer in inactive *ALDH2*2* heterozygotes compared with those homozygous for the active *ALDH2*1* allele (Yokoyama *et al.*, 1998a). The other four studies reported no overall association between *ALDH2* genetic polymorphism and colorectal cancer (Murata *et al.*, 1999; Matsuo *et al.*, 2002; Otani *et al.*, 2005; Matsuo *et al.*, 2006a), but one suggested that heterozygosity for inactive *ALDH2* increased the risk for colon cancer associated with alcoholic beverage consumption (Murata *et al.*, 1999), and another suggested that heterozygosity for inactive *ALDH2* increased the risk for rectal cancer associated with alcoholic beverage consumption (Matsuo *et al.*, 2002). One study examined the relationship between the composite *ALDH2* and *ADH1B* genotype and colorectal cancer (Matsuo *et al.*, 2006a). In this study, the combination of the *ALDH2*1/*1* and *ADH1B*1/*2* genotype as well as that of the *ALDH2*2* and *ADH1B*2/*2* allele was associated with a substantial decrease in the risk compared with *ALDH2*1/*1* and *ADH1B*2/*2*; adjusted odds ratios for individuals harbouring the *ALDH2*1/*1* genotype and the *ADH1B*1* allele, the *ALDH2*2* allele and the *ADH1B*2/*2* genotype, and the *ALDH2*2* allele and the *ADH1B*1* allele were 0.10 (95% CI, 0.04–0.21), 0.10 (95% CI, 0.06–0.19) and 1.36 (95% CI, 0.94–1.97), respectively. [The Working Group noted that interpretation of the findings was difficult with respect to etiological significance.] The associations with composite genotypes did not differ greatly by alcoholic beverage intake (Matsuo *et al.*, 2002). Two studies examined the relationship between *ALDH2* genotype and colorectal adenomas based on independent data sets in the Self Defence Forces Health Study (Takeshita *et al.*, 2000b; Hirose *et al.*, 2005). The first study was small in size (69 cases and 131 controls) and showed no difference in the distribution of genotypes between cases and controls. The second study was based on 452 cases of colorectal adenoma and 1050 controls; odds ratios for *ALDH*1/*1*, *ALDH*1/*2* and *ALDH*2/*2* were 1.00 (reference), 0.81 (95% CI, 0.62–1.05) and 0.67 (95% CI, 0.35–1.27), respectively, with adjustment for

age, hospital, rank, cigarette smoking and alcoholic beverage use (categorized as life-long non-use, former use and current use of <30, 30–59 or >60 mL alcohol per day). No clear interaction between alcoholic beverage intake and *ALDH2* genotype was noted; high alcoholic beverage intake was associated with an approximately 1.5-fold increase in the risk (odds ratio, 1.53; 95% CI, 1.01–2.32) regardless of *ALDH2*1/*2* genotype. [The Working Group noted that the available epidemiological evidence was rather suggestive of the lack of an effect of the heterozygous *ALDH2* genotype to increase the risk for colorectal cancer. This may reflect the fact that acetaldehyde levels in the colon are high due to microbial metabolism of ethanol, and *ALDH2* plays only a small role in controlling this concentration (see Section 4.1.2).]

Breast cancer

A case–control study of female breast cancer in the Republic of Korea did not show any *ALDH2*-associated risk, but drinking status was not described in detail and no adjustment was made for alcoholic beverage drinking (Choi *et al*., 2003). [The Working Group noted that the epidemiological evidence was insufficient to support an association between heterozygous *ALDH2* genotype and breast cancer.]

Effects of ALDH2 deficiency on acetaldehyde levels

An alcohol-challenge test showed 10–20 times higher acetaldehyde levels in saliva than in blood (Homann *et al*., 1997), and the same and subsequent studies showed that oral microflora forms acetaldehyde from ethanol and largely contributes to acetaldehyde levels in saliva (Homann *et al*., 1997, 2000a). After a moderate oral dose of ethanol, the salivary acetaldehyde levels of individuals with inactive *ALDH2* were two to three times those of individuals with active *ALDH2* (Väkeväinen *et al*., 2000). *ALDH2* activity in the upper aerodigestive tract is extremely weak (Yin *et al*., 1997), and inefficient degradation of acetaldehyde in the upper aerodigestive tract may increase the risk for acetaldehyde-associated carcinogenesis. Higher levels of acetaldehyde–DNA adducts have been demonstrated in Japanese alcoholics with inactive heterozygous *ALDH2* than in those with active *ALDH2* (Matsuda *et al*., 2006). Also, sister chromatid exchange (Morimoto & Takeshita, 1996) and micronuclei (Ishikawa *et al*., 2003) are more frequent in the lymphocytes of habitual alcoholic beverage drinkers with inactive heterozygous *ALDH2* than in those of habitual drinkers with active *ALDH2*. More data on the genotoxic effects of acetaldehyde are discussed in Section 4.7.

(b) Genes involved in folate metabolism

(i) *Folate metabolism and genetic polymorphisms*

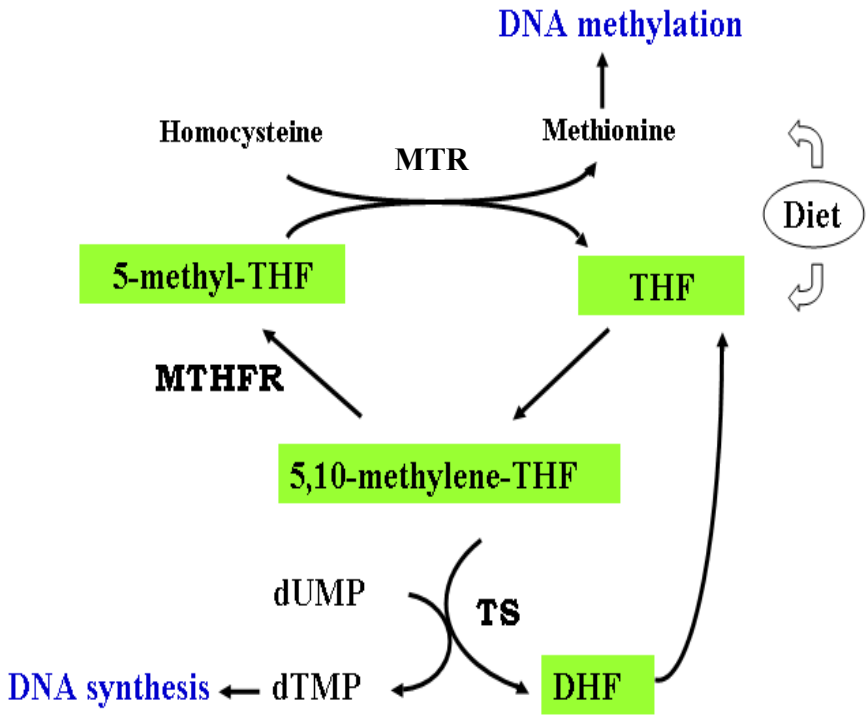
Excessive alcoholic beverage consumption causes folate deficiency, as exemplified by megaloblastic anaemia among alcoholics, and multiple effects of alcoholic beverages on folate metabolism have been described (Halsted *et al*., 2002; Mason & Choi, 2005). Alcoholic beverage consumption leads to folate depletion by decreasing its intestinal absorption and hepatic uptake and by increasing renal excretion through a reduction in tubular re-absorption; acetaldehyde also cleaves folate as shown *in vitro* by Shaw *et al*.

(1989). Acetaldehyde, rather than ethanol *per se*, was responsible for folate cleavage, although no such direct effect has been demonstrated in animals or humans (Mason & Choi, 2005). In a study of Japanese men in a rural community (Yokoyama *et al*., 2005), the amount of alcoholic beverage intake was not correlated with serum folate levels. An inverse correlation was found in carriers of the *ALDH2*1/*2* genotype, which renders the enzyme inactive, but not in those homozygous for the *ALDH2*1/*1* genotype.

Folate metabolism is linked to DNA methylation and synthesis, which are two crucial steps in carcinogenesis (Figure 4.3). Methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate-homocysteine *S*-methyltransferase (MTR) and thymidylate synthase (TS) are key enzymes in folate metabolism (Lucock, 2000; Mason & Choi, 2005), and genetic polymorphisms of these enzymes have been investigated widely, particularly in relation to the risk for colorectal cancer (Sharp & Little, 2004; Kono & Chen 2005). MTHFR irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group for the conversion of homocysteine to methionine, the precursor of *S*-adenosylmethionine, the universal methyl donor for methylation of a wide variety of biological substrates including DNA. MTR is a vitamin B12-dependent enzyme that catalyses the conversion of homocysteine to methionine. Depletion of methionine results in global genomic hypomethylation and aberrant methylation of CpG clusters in the promoters of tumour-suppressor and DNA-repair genes. The substrate of MTHFR, 5,10-methylenetetrahydrofolate, is required for TS-catalysed conversion of deoxyuridylate to thymidylate. An adequate supply of thymidylate is required for DNA synthesis and repair, and depletion of the thymidylate pool results in uracil misincorporation into DNA, leading to single- and double-strand breaks. Ethanol inhibits the reaction catalysed by MTR, resulting in a decrease in *S*-adenosylmethionine and genomic hypomethylation. Inhibition of the conversion of homocysteine to methionine also causes accumulation of 5-methyltetrahydrofolate (a substrate for MTR), i.e. the so-called 'methylfolate trap', and thereby depletes folate in the forms necessary for thymidylate synthesis (Mason & Choi, 2005).

Two functional common polymorphisms in the *MTHFR* gene have been determined. One is the *C677T* polymorphism, with an alanine-to-valine substitution at codon 222, which results in reduced activity of the enzyme, and the other is the *A1298C* polymorphism, which results in a substitution of glutamate with alanine at codon 429 (Frosst *et al*., 1995; van der Put *et al*., 1998). Lower activities of the enzyme are also noted in relation to the *MTHFR A1298C* polymorphism, although the extent of reduction is less evident (Weisberg *et al*., 1998). With regard to the *MTR* gene, the *A2756G* polymorphism that comprises a change from aspartate to glycine at codon 919 has been deemed functional in terms of serum homocysteine and folate levels (van der Put *et al*., 1997). A tandem-repeat polymorphism exists in the enhancer region of the *TS* promoter, which contains triple (*TS*3R*) or double (*TS*2*R) repeats of a 28-base-pair sequence (Horie *et al*., 1995); rare alleles containing larger repeats have also been documented (Matsuo *et al*., 2005). The expression of mRNA is enhanced in individuals who are homozygous for the triple repeats (*TS 3R/3R*) over those with the *TS 2R/2R*

Figure 4.3. Abbreviated scheme of folate metabolism in relation to DNA methylation and thymidylate synthesis



From Kono & Chen (2005)
dTMP, deoxythymidine monophosphate (deoxythymidylate); dUMP, deoxyuridine monophosphate (deoxyuridylate); MTHFR, methylenetetrahydrofolate reductase; MTR, 5-methyltetrahydrofolate-homocysteine S-methyltransferase (also called methionine synthase); THF, tetrahydrofolate; TS, thimidylate synthase

genotype (Trinh *et al.*, 2002). A second *TS* polymorphism, a 6-base-pair deletion in the 3′ untranslated region (*TS 1494del6*), is assumed to be associated with decreased mRNA stability (Ulrich *et al.*, 2000; Mandola *et al.*, 2004; Ulrich *et al.*, 2005).

(ii) *Cancers associated with folate metabolism status*

Colorectal cancer

Two case–control studies nested in the Health Professionals Follow-up Study and the Physicians' Health Study in the USA first reported a decreased risk for colorectal cancer associated with the *MTHFR 677TT* genotype (Chen *et al.*, 1996; Ma *et al.*, 1997). Several studies have replicated this initial finding in different populations, although some have failed to find such an association, as reviewed elsewhere (Sharp & Little, 2004; Kono & Chen, 2005). In a meta-analysis of 16 studies (Kono & Chen, 2005), the combined odds ratio for the *677TT* versus *677CC* genotype was 0.82 (95% CI, 0.72–0.93), while the corresponding value for the *677CT* genotype was 0.97 (95% CI: 0.90–1.04). Results from more recent studies are also consistent with the above estimates (Le Marchand *et al.*, 2005; Matsuo *et al.*, 2005). Thus, the *MTHFR 677TT* genotype has the potential to protect against colorectal cancer.

Results on the *MTHFR A1298C* polymorphism and colorectal cancer are variable across and within studies (Kono & Chen, 2005). In case-control studies in the USA, a decreased risk for colorectal cancer for *MTHFR 1298CC* versus *1298AA* was observed in whites, but not in blacks (Keku *et al.*, 2002), and in women, but not in men (Curtin *et al.*, 2004). No clear association between the *MTHFR 677TT* genotype and colorectal cancer was seen in these studies. The *MTHFR C677T* and *A1298C* polymorphisms are in linkage disequilibrium, and an independent effect of *1298CC* (or *677TT*) is only examined in individuals with the *677CC* (or *1298AA*) genotype. Decreased risk associated with the *677TT* genotype in those with the *1298AA* genotype is more consistent than decreased risk for the *1298CC* genotype in those with the *677CC* genotype (Kono & Chen, 2005). As only few studies are available, the role of the *MTHFRA 1298C* polymorphism in colorectal cancer is uncertain.

Decreased risk for colorectal cancer associated with *MTHFR 677TT* is typically observed in individuals with high folate intake (Giovannucci, 2004; Kono & Chen, 2005). Similarly, an evident decrease in the risk for colorectal cancer associated with the *MTHFR 677TT* genotype was seen more frequently in individuals with no or light consumption of alcoholic beverages (Table 4.9). Part of the inconsistency in the findings may be due to differences in the overall folate status among study populations. Alcoholic beverage intake is an important determinant of folate status in populations with folate-replete diets such as health professionals and physicians in the USA (Giovannucci, 2004). Because the production of 5-methyltetrahydrofolate (a substrate for MTR) is reduced in individuals with *MTHFR 677TT*, an increased rather than a decreased risk due to DNA hypomethylation is expected in carriers of this allele. It is now considered that low activity of MTHFR or the *677TT* genotype is probably advantageous as it ensures a thymidylate pool for DNA synthesis when folate status is replete

Table 4.9 Odds ratios (and 95% confidence intervals [CI]) for colorectal cancer for the *MTHFR 677TT* genotype in combination with alcoholic beverage consumption

Reference, study location and period	Sex	Alcohol intake	Odds ratio (95% CI)	*p* for interaction
Chen *et al.* (1996), USA, 1986–94	Men	Low (≤1 drinks/week) Medium High (≥5 drinks/week)	0.11 (0.01–0.85) 0.55 (0.18–1.64) 1.56 (0.65–3.81)	0.02
Ma *et al.* (1997), USA, 1982–95	Men	Low (0–0.14 drinks/day) Medium (0.15–0.8 drinks/day) High (≥0.9 drinks/day)	0.12 (0.03–0.57) 0.42 (0.15–1.20) 1.31 (0.48–3.58)	<0.01
Slattery *et al.* (1999), USA, 1991–94	Both	Low (≤1 g/day) Medium High (>20 g/day)	1.0 (0.7–1.4) 0.5 (0.3–0.8) 1.0 (0.6–1.6)	Not reported
Keku *et al.* (2002), USA, 1996–2000	Both	Never Ever	1.0 (0.5–2.1) 0.7 (0.3–1.4)	
Yin *et al.* (2004), Japan 2000–03	Both	None Medium (<1 unit/day) High (≥1 unit/day)	0.58 (0.36–0.93) 0.73 (0.40–1.33) 0.89 (0.53–1.47)	0.62
Le Marchand *et al.* (2005), USA, 1995–99	Both	≤ Median (0.01 g ethanol/day) > Median	0.53 (0.34–0.82) 1.06 (0.74–1.56)	0.02
Matsuo *et al.* (2005), Japan 2001–04	Both	None Medium High (≥5 drinks/week, 50 g ethanol/drink)	1.48 (0.70–2.78) 0.51 (0.24–1.09) 0.43 (0.12–1.57)	Not reported

MTHFR, methylene tetrahydrofolate reductase The reference category is the *MTHFR 677CC* or *CC/CT* genotype with the lowest level of alcoholic beverage consumption. The *CC* and *CT* genotypes were combined in studies by Chen *et al.* (1996), Yin *et al.* (2004) and Le Marchand *et al.* (2005).

(Chen *et al.*, 1996; Giovannucci, 2004). Studies of colorectal adenoma have generally failed to showed an inverse association between the *MTHFR C677T* polymorphism and overall risk, but suggested that risks associated with the *MTHFR 677TT* genotype were differential according to folate or alcoholic beverage intake; the risk was elevated in those who had high alcoholic beverage or low folate intake and was decreased in those with low alcohol or high folate intake (Levine *et al.*, 2000; Ulvik *et al.*, 2001; Giovannucci *et al.*, 2003; Marugame *et al.*, 2003). In the case of folate depletion, the *MTHFR 677TT* genotype may diminish DNA methylation due to a decrease in methionine synthesis (Friso *et al.*, 2002; Giovannucci, 2004).

The variant homozygote (*GG*) of the *MTR A2576G* polymorphism was related to a decreased risk for colorectal cancer, especially in subjects with low alcoholic beverage consumption (<1 drink/day), in the combined analysis of the Physicians' Health

Study and the Health Professionals Follow-up Study (Ma *et al.*, 1999). A decreased risk for colorectal cancer associated with *2576GG* was also noted in Norway (Ulvik *et al.*, 2004), but not in other studies in the USA (Le Marchand *et al.*, 2002; Ulrich *et al.*, 2005) or Japan (Matsuo *et al.*, 2005). There was even an increased risk associated with the *2576GG* genotype in alcoholic beverage drinkers in the Japanese study (Matsuo *et al.*, 2005). A study of colorectal adenoma suggested an increased risk in women, but not in men, who had the *2576G* allele and high alcoholic beverage consumption (Goode *et al.*, 2004).

Individuals homozygous for double repeats of the *TS* enhancer region (*TS 2R/2R*) consistently show a decreased risk for colorectal cancer compared with those with the *TS 3R/3R* genotype (Chen *et al.*, 2003; Ulrich *et al.*, 2005; Matsuo *et al.*, 2005). While the *TS*-repeat polymorphism was unrelated to the overall risk for colorectal adenoma (Ulrich *et al.*, 2002; Chen *et al.*, 2004), those with high *TS* expression (*TS 3R/3R*) showed a threefold increase in risk only when they had high alcoholic beverage consumption (Chen *et al.*, 2004). Similarly, the risk for adenoma for the *TS 3R/3R* versus *2R/2R* genotype was elevated when folate intake was low, but was lowered when folate intake was high (Ulrich *et al.*, 2002). No clear association was observed for the *TS 1494del6* polymorphism in relation to colorectal cancer and adenoma (Ulrich *et al.*, 2002; Chen *et al.*, 2003; Ulrich *et al.*, 2005).

Other cancers

Studies on the *MTHFR C677T* polymorphism and the risk for breast cancer have produced rather mixed results. In a meta-analysis of 15 cases–control studies and two cohort studies (Lewis *et al.*, 2006), the authors reported an odds ratio of 1.04 (95% CI, 0.96–1.16) for the *677TT* versus the *677CC* genotype. In the Shanghai Breast Cancer Study (Shrubsole *et al.*, 2004) and the Long Island Breast Cancer Study (Chen *et al.*, 2005b), the authors found an increased risk associated with the *MTHFR 677TT* genotype among women with low folate intake. In a case–control study nested within the Multiethnic Cohort Study (Le Marchand *et al.*, 2004), the *MTHFR 677TT* genotype was associated with a decreased risk for breast cancer in women who had ever used hormone replacement therapy. In this subgroup, a decreased risk for the *677TT* genotype was noted in women with low alcoholic beverage consumption. The *MTHFR A1298C* polymorphism itself does not seem to be associated with risk for breast cancer (Shrubsole *et al.*, 2004; Le Marchand *et al.*, 2004; Chen *et al.*, 2005b; Justenhoven *et al.*, 2005). Justenhoven *et al.* (2005) examined the association of the *MTR A2756G* and *TS 1494del6* polymorphisms and found no clear association with the risk for breast cancer.

In a recent meta-analysis of the relationship between the *MTHFR C677T* polymorphism and the risk for oesophageal, gastric and pancreatic cancer (Larsson *et al.*, 2006), the investigators reported combined odds ratios associated with the *677TT* genotype compared with the *677CC* genotype of 1.90 (95% CI, 1.38–2.60) for gastric cardia adenocarcinoma based on four studies in China and one study in Italy and of 1.68 (95% CI: 1.29–2.19) for gastric cancer at all sites based on three studies in China and

one study each in Italy, Mexico and the Republic of Korea. Results for oesophageal squamous-cell carcinoma in seven populations (five in China and one each in Japan and Germany) and for pancreatic cancer in three studies were highly heterogeneous, and combined odds ratios were not estimated for these cancers. A limited number of studies suggested a greater increase in risk associated with the *MTHFR 677TT* genotype for gastric cardia carcinoma (Stolzenberg-Solomon *et al.*, 2003) and for pancreatic cancer (Li *et al.*, 2005b; Wang *et al.*, 2005b) among alcoholic beverage drinkers. In contrast, the *MTHFR 677CC* genotype was associated with an increased risk for hepatocellular carcinoma in patients with alcoholic liver cirrhosis (Saffroy *et al.*, 2004). Defective DNA synthesis may also play an important role in alcohol-related carcinogenesis in a folate-deficient state.

(c) Genes involved in DNA repair

Several studies have investigated the possible role of DNA-repair gene variants in carcinogenesis associated with alcoholic beverage consumption. In contrast to the strong effects of *ADH* and *ALDH* variants, the reported effects of DNA-repair gene variants have been quite modest and of borderline significance. In a recent review, Boffetta and Hashibe (2006) reported "small but insignificant differences in risk between current drinkers and non-drinkers for sequence variants in *XRCC1, OGG1, XPC* and *ERCC2*".

Below is a summary of the studies, divided by the repair pathway and directly related to alcoholic beverage drinking.

(i) *Direct repair by O^6-methylguanine methyltransferase (MGMT)*

Genetic variation in *MGMT* is of interest in view of earlier findings that exposure to ethanol decreases the activity of this repair enzyme in rats (Garro *et al.*, 1986; Wilson *et al.*, 1994). Two *MGMT* polymorphisms have been studied primarily: *Leu84Phe* and *Ile143Val*. Huang *et al.* (2005) found that *Phe84* and *Val143* alleles were protective against head and neck cancers. Notably, the protective effect of *Val143* was particularly pronounced in alcoholic beverage drinkers who consumed more than 21 drinks per week. However, these authors had noted that the same allele was associated with an increased risk for lung cancer in an earlier, smaller study. Tranah *et al.* (2006) investigated the relationship between the same *MGMT* variants and colorectal cancer. These authors found that the *Leu84* allele interacted with alcoholic beverage consumption, but only in women. They suggested that this effect involves an interaction of MGMT with the estrogen receptor rather than an effect on DNA repair. Studies by Teo *et al.* (2001) have shown that, following the removal of an O^6-methylguanine adduct, the modified MGMT enzyme can prevent the estrogen receptor-stimulated gene expression that is important for cell proliferation. Indeed, the *MGMT 84 Phe/Phe* genotype is associated with an increased risk for breast cancer in postmenopausal women (Han *et al.*, 2006), although until now there is no evidence of an interaction with alcoholic beverage drinking.

(ii) *Base-excision repair*

The *Ser321Cys* variant of the 8-oxoguanine DNA glycosylase 1 (*OGG1*) gene has been identified in the human population. *OGG1* encodes a DNA glycosylase that is responsible for the first step in the repair of the oxidative DNA lesion 8-oxo-deoxyguanine. One study suggests that the Cys-containing enzyme is significantly less active than the Ser-containing form (Kohno *et al.*, 1998). Takezaki *et al.* (2002) observed no effect of the *OGG1* genotype on the overall odds ratio for stomach cancer; however, in individuals who drank more than two drinks per week, the odds ratio for the *Cys/Cys* genotype was 6.55 (95% CI, 1.21–35.5). Elahi *et al.* (2002) also found that the *OGG1 Cys* allele was associated with an increased risk for orolaryngeal cancer. Stratifying by drinking behaviour, they found no association between genotype and cancer in never drinkers, but an increased risk for cancer in alcoholic beverage drinkers homozygous for the *Cys* allele.

(iii) *Nucleotide-excision repair*

The nucleotide-excision-repair pathway may play a role in the repair of several types of DNA lesion that could result from alcoholic beverage consumption or acetaldehyde, such as the malondialdehyde–deoxyguanine and crotonaldehyde–deoxyguanine adducts (Brooks & Theruvathu, 2005; Theruvathu *et al.*, 2005; Matsuda *et al.*, 2006). Shen *et al.* (2001) found that individuals who carry the +/+ genotype for a xeroderma pigmentosum (XP) complementation group C-biallelic poly(AT) insertion/deletion (*XPC-PAT)* intronic polymorphism had a slightly increased risk for head and neck cancer, and that this genotype was associated with an increased risk in never drinkers and former drinkers, but not in current drinkers. Sturgis *et al.* (2000) focused on the *XPD* polymorphism *Gln751Lys*, and found that the *Lys/Lys* genotype was associated with an increased risk for head and neck cancers, and that the risk for this genotype was higher in current tobacco smokers and current alcoholic beverage drinkers. [It should also be pointed out that, although the *XPD Lys751Gln* is commonly considered to be a functional polymorphism, there is little direct evidence to support this, and both functional and evolutionary evidence suggest that this polymorphism is in fact benign (Clarkson & Wood, 2005).]

Cui *et al.* (2006) studied the relationship between the *XPG His1104Asp* polymorphism and lung cancer and squamous-cell carcinomas of the larynx and oesophagus in relation to alcoholic beverage drinking and smoking. They found an increased risk for squamous-cell carcinomas in heavy drinkers who had at least one copy of the *His* allele. [In contrast to the *Gln751Lys* polymorphism, the *His1104Asp* polymorphism is probably functional, based on evolutionary considerations.]

(iv) *Single-strand break repair*

The single-strand break-repair pathway may be particularly important in protecting against DNA damage that results from alcoholic beverage intake, because several studies with the comet assay have shown that exposure of cells to ethanol *in vitro*

can cause single-strand breaks (Blasiak *et al*., 2000; Eysseric *et al*., 2000; Lamarche *et al*., 2003, 2004). However, the relationship between single-strand breaks and cancer is obscured by the fact that patients with a defect in the repair of single-strand breaks develop neurological disease, but are not at significantly increased risk for cancer (Caldecott, 2003).

Kietthubthew *et al*. (2006) found a marginally significant risk for oral cancer with the X-ray repair cross-complementing group 1 *(XRCC1) 194Trp* allele, and reported that this allele interacted with alcoholic beverage and tobacco consumption to increase this risk. With regard to the *XRCC1 Arg399Gln* variant, Sturgis *et al*. (1999) observed a significantly increased risk associated with the *Gln/Gln* genotype among current users of tobacco and alcoholic beverages. In contrast, Lee *et al*. (2001) observed that the *Arg/Arg* genotype was associated with an increased risk for oesophageal cancer in alcoholic beverage drinkers, but not in non-drinkers. Finally, Hong *et al*. (2005) determined the genotypes for three *XRCC1* polymorphisms (*Arg194Trp*, *Arg399Gln* and *Arg280His*) in colorectal cancer patients and non-cancer controls. Certain combinations of these genotypes altered the risk for colorectal cancer in subjects who drank >80 g ethanol per week.

4.3.2 *Experimental systems*

Błasiak (2001) found that exposure of human lymphocytes to 30 mM ethanol inhibited the repair of DNA strand breaks generated by the radiomimetic drug bleomycin. Pool-Zobel *et al*. (2004) used the comet assay to study DNA damage and repair in cells obtained from rectal biopsies from human alcoholic beverage abusers and controls. They found that DNA damage in these cells correlated with DNA damage in lymphocytes. Male alcoholic beverage abusers had significantly less damage than controls, and their cells showed greater repair than those of controls following exposure of the cells to hydrogen peroxide. The authors proposed that this may be the result of an induction of repair as a result of the alcoholic beverage abuse.

Asami *et al*. (2000) exposed rats to increasing concentrations of ethanol (12–70%) in the drinking-water over a 20-week period. When concentrations of ethanol reached 50%, one group of rats was switched from a standard diet to an autoclaved diet to simulate nutrient deficiency. Groups of rats were killed at various time points, and the levels of 8-oxo-deoxyguanine and the activity of its repair enzyme in oesophageal mucosa were assayed. Levels of both 8-oxo-deoxyguanine and repair-enzyme activity were increased by feeding the autoclaved diet. Ethanol had no effect alone, but potentiated the effect of the autoclaved diet. [As this is a very unusual experimental model, it is difficult to draw any conclusions from this study.]

Bradford *et al*. (2005) found that rats and mice exposed to ethanol (35% of calorie intake) via intragastric feeding showed increased levels of oxidative DNA damage, as well as an increased expression level of base excision-repair in the liver, which suggested a compensatory induction of base excision repair by ethanol. These effects

were not seen in *CYP2E1* knockout mice, and were blocked by a CYP2E1 inhibitor. Navasumrit *et al.* (2001a) observed a decrease in hepatic MGMT activity after a single intragastric dose of ethanol (5 g/kg), which is consistent with earlier findings that either acute or chronic treatment with ethanol reduced the activity of this enzyme (Garro *et al.*, 1986; Wilson *et al.*, 1994). The activities of other base excision-repair enzymes, alkylpurine-DNA–*N*-glycosylase and OGG1, were also modulated by treatment with ethanol. Four weeks of feeding a liquid diet (36% ethanol-derived calories) decreased alkypurine-DNA-*N*-glycosylase activity, whereas OGG1 activity was elevated after 1 week of ethanol in liquid diet, but decreased after 4 weeks (Navasumrit *et al.*, 2001a).

4.4 Modifying effects of ethanol consumption on metabolism and clearance

4.4.1 *Humans*

The metabolism and clearance of ethanol are relevant to tumorigenesis in several regards: effects on the level and time course of exposure of target tissues to ethanol; the generation of toxic by-products, particularly reactive oxygen species, during metabolism; and the derangement of other metabolic pathways as a result of co-factor depletion and alteration of intracellular and extracellular signalling.

(a) Effects of ethanol on ethanol metabolism

Ethanol is metabolized by ADH, CYP2E1, -1A2 and -3A4, catalase and, in certain tissues, the non-oxidative free fatty acid ethyl ester synthases (FAEES). ADHs have a higher affinity for ethanol than the CYPs, and are present in substantial quantities in the liver; they provide the major route for catabolism of low-to-moderate concentrations of ethanol (reviewed in Crabb, 1995; Lieber, 1999; Agarwal, 2001; Lieber, 2004a; Gemma *et al.*, 2006). ADH is induced in rat liver *in vivo* by intoxicating concentrations of ethanol (Badger *et al.*, 2000; Wang *et al.*, 2002), but this has not been confirmed for humans.

Hepatic microsomal CYP2E1 plays an increasingly important role as blood ethanol concentrations rise, and degrades a significant percentage (up to 10%) of ingested ethanol (reviewed in Fraser, 1997; Gemma *et al.*, 2006). Regulation of CYP2E1 by ethanol is complex and may involve transcriptional, post-transcriptional, translational and post-translational mechanisms (reviewed in Lieber, 1999; Novak & Woodcroft, 2000; Lieber, 2004a; Gonzalez, 2007). CYP2E1 is induced by ethanol in human liver and in cultured liver cells (reviewed in Crabb, 1995; Novak & Woodcroft, 2000; Cederbaum, 2006; Gonzalez, 2007). Induction may occur with a single, moderately high dose (0.8 g/kg bw) (Loizou & Cocker, 2001). In recently drinking alcoholics, CYP2E1 in liver samples was increased fourfold compared with the level in non-drinkers (Tsutsumi *et al.*, 1989), which is in line with an about threefold higher rate of clearance of chlorzoxazone, a CYP2E1 substrate, in alcoholics. The half-life of CYP2E1 was reported to be 2.5 days in abstaining alcoholics (Lucas *et al.*, 1995). Immunohistochemistry

revealed that the hepatic induction of CYP2E1 was primarily perivenous (centrilobular). In the livers of alcoholics, midzonal as well as perivenular CYP2E1 protein was increased and this increase was strongly correlated with elevated *CYP2E1* mRNA (Takahashi *et al.*, 1993).

There is evidence that the isoenzymes, CYP1A2 and CYP3A4 may be induced by alcohol *in vivo*. In alcoholics, the metabolism of certain drugs that are metabolized by CYPs other than CYP2E1 showed increased clearance, although the complexity of factors and conditions do not allow firm conclusions to be drawn (reviewed by Klotz & Ammon, 1998; Sinclair *et al.*, 1998). With the use of midazolam as an indicator of CYP3A activity, individuals with moderate alcoholic beverage consumption (2–3 drinks/day) did not show a difference in systemic clearance, but maximum serum concentration and oral availability differed; there was evidence of induction of CYP3A in the small bowel (Liangpunsakul *et al.*, 2005).

(b) Effects of ethanol on clearance of ethanol from tissues and organisms

The clearance of ethanol is determined primarily by ADH (see Section 4.1). Of the purified ADH alloenzymes, all but ADH1B3, ADH3 and ADH4 are inhibited by ethanol (Lee *et al.*, 2006), which could impede the clearance of ethanol by either the stomach or liver. In addition, ADH in the stomach is decreased in instances of gastritis and gastric atrophy (Brown *et al.*, 1995), such as those induced by alcohol intoxication. ADH was reduced in the gastric mucosa of young male alcoholics (Seitz *et al.*, 1993) and in men of various ages as a function of daily alcoholic beverage intake (Parlesak *et al.*, 2002). The increase in gastric ADH in alcoholics during abstinence from alcohol was interpreted as evidence of its suppression during alcoholic beverage use (Watanabe, 1997). In addition, young women had lower levels of gastric ADH compared with men of the same age (Seitz *et al.*, 1993). Gastric ADH was lower in alcoholic men and women than in non-alcoholics and correlated with reduced first-pass clearance of ethanol in one study (Frezza *et al.*, 1990). In other investigations no correlation was found between first-pass metabolism of ethanol and gastric ADH (Brown *et al.*, 1995) or gastritis in elderly subjects (Pedrosa *et al.*, 1996).

In addition to inducing CYP2E1, ethanol is a very effective competitive inhibitor of CYP2E1 in humans, as assessed by clearance of chlorzoxazone, a CYP2E1 substrate: an acute dose of 0.8 g/kg bw ethanol reduced chlorzoxazone metabolism by 94% (Loizou & Cocker, 2001). Ethanol may also reduce CYP2E1 indirectly as a result of alcoholic liver disease (Dilger *et al.*, 1997). There is evidence that alcoholism reduces first-pass clearance of ethanol (reviewed in Caballería, 1992). When non-alcoholics and alcoholics consumed 150 mg/kg bw ethanol, the first-pass metabolism accounted for 73% and 23% in these groups, respectively (DiPadova *et al.*, 1987). It is probable that part of this effect can be attributed to the direct or indirect actions of ethanol.

Polymorphisms in *ADH* and *CYP2E1* did not relate to gastrointestinal symptoms in alcoholics (Laheij *et al.*, 2004). *ADH* polymorphisms were investigated in the context

of first-pass ethanol metabolism and levels of gastric ADH; individuals who were homozygous for ADH_3^1 (*ADH1C*1*) presented greater ADH activity in gastric biopsies and more rapid clearance than those who were homo- or heterozygous for ADH_3^2 (*AHD1C*2*) (Oneta *et al*., 1998). Although these differences were not statistically significant due to small group-sizes, they were consistent with the higher V_{max} for the *ADH1C*1* form (reviewed in Crabb, 1995).

The rate of gastric emptying also has a major effect on first-pass clearance by the liver (Oneta *et al*., 1998), since a slow rate of delivery of ethanol to the low-K_m hepatic ADHs favours more complete metabolism. Alcoholic beverages as well as various drugs may alter the bioavailability of ethanol via their effects on gastric emptying (Pfeiffer *et al*., 1992; Fraser, 1997); pure ethanol and whisky caused a delay and beer accelerated the process. Mixed findings were reported for white wine. Variations in ethanol concentration, osmolarity and caloric content are thought to contribute to this discrepancy (Pfeiffer *et al*., 1992). Gastric emptying was accelerated by the consumption of ethanol during a meal (Wedel *et al.,* 1991). In contrast, among 46 chronic alcoholics, 11 (23.9%) showed delayed gastric emptying in association with high ethanol consumption and dyspeptic symptoms, and all alcoholics showed an increased mouth-to-caecum transit time (Wegener *et al*., 1991).

In summary, ethanol and/or the constituents of alcoholic beverages may influence the metabolism of ethanol in humans by specific induction of CYP2E1 and -3A4 and possibly -1A2; by competitive inhibition of CYP2E1 activity in the liver, direct inhibition of ADHs in the liver and gastric mucosa, toxic effects on the gastric mucosa that cause loss of ADH, possible induction of hepatic ADH at high doses and by effects on gastric emptying, which may be variable and complex.

(c) Effects of ethanol on the metabolism of xenobiotics

Ethanol interacts with the metabolism of xenobiotics, mainly through the CYP enzymes, in at least two distinct ways: by the induction of metabolic activation leading to enhanced formation of proximate reactive chemical species; and by competitive inhibition of metabolism and clearance, such that central hepatic and gastointestinal clearance is reduced, which results in increased dose delivery to peripheral target tissues (reviewed in Meskar *et al*., 2001). Alteration of phase II conjugation/detoxification enzymes by ethanol may also occur, but this has been studied less extensively.

(d) Effects of ethanol via the induction of CYP2E1

As noted above, ethanol induces CYP2E1 in human liver. Among more than 70 substrates of CYP2E1 (Raucy *et al*., 1993; Guengerich *et al*., 1994; Djordjević *et al*., 1998; Klotz & Ammon, 1998; Cederbaum, 2006) are known carcinogens such as benzene, butadiene and vinyl chloride, as well as many other compounds, e.g. acrylonitrile, azoxymethane, chloroform, carbon tetrachloride, methylazoxymethanol and trichloroethylene. Increased toxicity results from the metabolism of many of these

chemicals induced by CYP2E1. For example, pyridine, a constituent of tobacco smoke, is a substrate of CYP2E1 that generates redox cycling, which leads to DNA damage (reviewed in Novak & Woodcroft, 2000).

In humans, in addition to the prominent expression of CYP2E1 in the perivenous (centrilobular) regions of the liver, the enzyme is also detectable in the kidney cortex and, at lower levels, in the oropharynx, nasal mucosa, ovary, testis, small intestine, colon, pancreas, endothelial cells of the umbilical vein and in lymphocytes (reviewed in Ingelman-Sundberg *et al.*, 1994; Lieber, 1999, 2004a). This enzyme may thus participate in the genesis of cancers at several important target sites. In the liver, induction of CYP2E1 by ethanol has been demonstrated both *in vivo* and in primary hepatocytes (see below). Levels of hepatic CYP2E1 in humans vary at least 50-fold, which is assumed to be due to various inductive influences that possibly interact with polymorphisms in gene regulatory regions (reviewed in Ingelman-Sundberg *et al.*, 1994). Induction of CYP2E1 in extrahepatic tissues has not been studied extensively in humans. Levels of *CYP2E1* mRNA and protein in the lymphocytes of heavy alcohol drinkers correlated well with clearance rates for chlorzoxazone, a marker for hepatic CYP2E1 (Raucy *et al.*, 1997, 1999). This correlation was not seen in a study of moderate alcoholic beverage drinkers (Liangpunsakul *et al.*, 2005).

(e) Effects of induction of other xenobiotic-activating CYPs by ethanol

As noted above, several CYPs in addition to CYP2E1 may be induced by ethanol. Of particular interest are CYP1A2, which activates heterocyclic amines (Oda *et al.*, 2001), and the enzymes in the CYP3A family, which have wide substrate specificity and have been implicated in the activation of several known or suspected human carcinogens, including aflatoxin (IARC, 2002; Kamdem *et al.*, 2006). Although the affinity is low, both isoforms metabolize the tobacco carcinogen, NNK (Jalas *et al.*, 2005). In humans with moderate alcoholic beverage consumption, the possible induction of CYP3A in the intestine was inferred from the reduced oral bio-availability of midazolam (Liangpunsakul *et al.*, 2005).

(f) Effects of inhibition of CYPs by ethanol

Ethanol is a competitive inhibitor of CYP2E1 (Anderson, 1992). At a concentration of 1%, it inhibits the activities of CYP1A1, -2B6 and -2C19 expressed from transfected genes in cultured human lymphoblastoid cells. In this system, ethanol (1%) did not inhibit the activity of CYP1A2, -2C8, -2C9 or -3A4 (Busby *et al.*, 1999). Other studies also showed no inhibition of CYP3A by ethanol (Feierman *et al.*, 2003). There is indirect evidence that ethanol can inhibit the first-pass hepatic metabolism of the environmental carcinogen NDMA in humans, allowing release of this compound into the blood: individuals with chronic renal failure showed detectable blood and urine levels of NDMA, which were increased by consumption of ethanol (Dunn *et al.*, 1990).

4.4.2 *Experimental systems*

Most studies on the in-vivo effects of ethanol in animals have used rats. These experiments involved either pair-feeding of liquid diet with ethanol as 35% of the caloric intake (Lieber-DiCarli model) or gastric infusion of a liquid diet (total enteric nutrition) to achieve blood levels of ethanol comparable with those in human alcoholics, and to induce hepatotoxicity. These modes of exposure are hereafter referred to as LDC and TEN diets, respectively. The TEN model has been shown to maintain normal body weights of the animals, whereas general health effects, including weight loss, may result from feeding the LDC-type diet (Badger *et al*., 1993).

(a) Effects of ethanol on ethanol metabolism

Similarly to humans, involvement of ADH and CYP2E1 in the metabolism and clearance of ethanol has been confirmed in animals (Gonzalez, 2007). During continuous feeding of rodents with ethanol via intragastric infusion, the blood ethanol levels vary in a cyclic manner (Tsukamoto *et al*., 1985), which suggests that rates of metabolism change independently of the uptake of ethanol. Recent data (reviewed by French, 2005) suggest that this phenomenon is directly linked to the liver toxicity of ethanol and depends on the proper functioning of the intact hypothalamic–pituitary–thyroid axis (Li *et al*., 2000), the release of norepinephrine (Li *et al*., 2003) and the availability of cofactors such as NAD to support the oxidation of ethanol by ADH (Bardag-Gorce *et al*., 2002). Changes in hepatic ADH and in the expression of CCAAT/enhancer-binding proteins and of sterol regulatory element-binding protein 1 (SREBP-1) as a result of continuous infusion of ethanol-containing diets into rats have also been studied (Badger *et al*., 2000; He *et al*., 2002, 2004). Induction of hepatic ADH was demonstrated in a rat model that involved repeated intragastric treatment with acute doses of ethanol, which resulted in progressive pathological changes in both the liver and gastric mucosa. A reduction in gastric ADH occurred concomitantly with an increase in hepatic ADH (Wang *et al*., 2002). However, with an LDC-type diet, gastric ADH did not change, although microsomal ethanol metabolism increased significantly (Pronko *et al*., 2002). ADH may also be influenced indirectly by ethanol suppression of testosterone, which reduces the expression of hepatic ADH in spontaneously hypertensive rats (Rachamin *et al.,* 1980).

In rats and rabbits, CYP2E1 contributed 10% and 40–50% of ethanol clearance at 10 mM and 100 mM ethanol, respectively (Fujimiya *et al*., 1989; Matsumoto *et al*., 1994; Matsumoto *et al*., 1996; Matsumoto & Fukui, 2002). Dietary composition can influence the induction of CYP2E1 in rat liver, and high-fat/low-carbohydrate diets produce the greatest induction, especially with unsaturated fat (Yoo *et al*., 1991; Lieber, 1999, 2004b; Cederbaum 2006). In rats given ethanol in a liquid diet, CYP2E1 was increased ninefold in liver microsomes and accounted for about 50% of CYP-dependent microsomal oxidation of ethanol (Johansson *et al*., 1988). Increased transcription of the *CYP2E1* gene appears to occur only at high doses: when rats received continuous

intragastric infusion with ethanol in a TEN liquid diet, hepatic CYP2E1 protein was induced at most doses tested, but mRNA increased only at urinary alcohol concentrations above 3 g/L (65 mM) (Ronis *et al.*, 1993). *CYP2E1* gene transcription in the liver is controlled by the HNF 1α transcription factor, as well as at least one other pathway that involves β-catenin (reviewed in Gonzalez, 2007). *CYP2E1* mRNA can also be destabilized and its rate of translation affected by insulin (De Waziers *et al.*, 1995).

CYP2E1 protein may be increased via enhanced transcription but also by upregulation of protein synthesis or by enhanced stability of the protein to degradation by the lysosomal or proteasomal pathways, which are influenced by substrate binding (reviewed in Gonzalez, 2007). Chronic administration of high doses of ethanol suppressed proteasome activity (Fataccioli *et al.*, 1999; Cederbaum, 2006). With an LDC diet, increased CYP2E1 protein was shown to be due to enhanced enzyme synthesis (Tsutsumi *et al.*, 1993) or protein stabilization by reduced ubiquitin–proteasome-catalysed degradation (Roberts *et al.*, 1995a). These effects are possibly dependent on the difference in age and/or size of the male Sprague-Dawley rats in these two studies (150–170 g and 100–120 g, respectively), because the hormonal status of rats changes markedly over this range.

CYP2E1 induction has also been studied in primary cultures of rat-liver hepatocytes and in FGC-4 rat hepatoma cells (McGehee *et al.*, 1994). A five- to sixfold maximal induction was observed at 10 mM ethanol, which was due to increased protein stability, with no increase in mRNA, as was also reported for human hepatoma cells. It was suggested that the increase in *CYP2E1* mRNA seen *in vivo* with high concentrations of ethanol may involve effects of hormones and other factors that are not present in cell cultures (reviewed in Novak & Woodcroft, 2000; Raucy *et al.*, 2004).

Ethanol also induced CYP3A in rat-liver cells and in intact rats (Feierman *et al.*, 2003), and CYP2B was induced both at the RNA and at the protein level in intact rats. However, the latter enzyme did not appear to contribute to the oxidation of ethanol (Johansson *et al.*, 1988; Sinclair *et al.*, 1991).

The relative contribution of catalase to the overall metabolism of ethanol is not fully resolved and may be more important in the brain than in the liver. The effects of catalase are greatest at high levels of ethanol and are dependent on concentrations of hydrogen peroxide. Rat hepatic catalase is increased moderately by chronic exposure to ethanol (Quertemont, 2004).

(b) Effects of ethanol on clearance of ethanol from tissues and organisms

Studies with baboons, rats and mice have engendered a debate on the relative importance of gastric and hepatic ADH in the first-pass clearance of ethanol. In baboons, the oesophageal mucosa contains higher ADH activity than the stomach, and the upper gastrointestinal tract provides the greatest contribution to first-pass metabolism (Baraona *et al.*, 2000). In rodents, different studies have concluded that first-pass metabolism of ethanol is predominately gastric (Lim *et al.*, 1993) or that gastric first-pass metabolism

is negligible (Pastino *et al*., 1996; Levitt *et al*., 1997b). Physiologically-based pharmacokinetic modelling indicated that gastric clearance was not important in mice (Pastino *et al*., 1996), but in rats the gastric first-pass metabolism cleared 26% and the hepatic metabolism cleared 12% of a 500-mg/kg dose of ethanol (Pastino & Conolly, 2000). At higher doses of ethanol, the relative importance of gastric clearance increased.

(c) Effects of ethanol via induction of CYP2E1

Regulation of CYP2E1 expression by ethanol is complex, and, as shown in rodent studies, may involve increased gene transcription, mRNA stability, translational efficiency or protein degradation (reviewed in Novak & Woodcroft, 2000). In-vitro studies of molecular regulation in humans have been limited to the use of primary hepatocytes and human hepatoma (HepG2) cells that stably express transfected CYP2E1. The induction of *CYP2E1* mRNA was increased twofold in cultured primary human hepatocytes by 50 mM ethanol, but no significant increase in protein was observed (Raucy *et al*., 2004). However, in HepG2 cells, ethanol induced CYP2E1 protein but not mRNA (reviewed in Lieber, 1999; Cederbaum, 2006). Inductive effects were maximal over a concentration range of 5–100 mM ethanol (Carroccio *et al*., 1994) and apparently involved inhibition of CYP2E1 protein degradation by the proteasome pathway (Cederbaum, 2006).

Ethanol is metabolized *in vitro* by human CYP1A2 and -3A4, as well as by CYP2E1, although with a somewhat lower catalytic efficiency (Salmela *et al*., 1998). The use of specific inhibitors in 18 human liver samples indicated that CYP2E1 contributed most to the oxidation of ethanol, while CYP1A2 and CYP3A4 together equalled CYP2E1 in activity (Salmela *et al*., 1998).

In cultured human HepG2 hepatoma cells, ethanol induced the expression of CYP3A4 from a transfected vector (Feierman *et al*., 2003). Isopentanol, which is a major higher-chain alcohol in beverages, synergized with ethanol to induce CYP3A in rats *in vivo* (Louis *et al*., 1994). In primary cultures of human hepatocytes, isopentanol induced CYP2E1 and particularly CYP3A4 (Kostrubsky *et al*., 1995). In addition, ethanol caused proliferation of the smooth endoplasmic reticulum, so that the levels of all CYP isoforms expressed there were increased (reviewed in Lieber, 2004a).

In addition to its well established effects in the liver, ethanol also induces CYP2E1 in extrahepatic tissues of animals. This may be particularly relevant to the activation of xenobiotics. In rats given ethanol in an LDC-type liquid diet, CYP2E1, as indicated by immunohistochemical staining, was increased in duodenal and jejunal villi and, in contrast to controls, could be detected in the squamous epithelium of the cheek mucosa, tongue, oesophagus and forestomach and in the surface epithelium of the proximal colon. The epithelium of the fundic and antral mucosa of the stomach, the ileum, the distal colon and the rectum remained negative for CYP2E1 (Shimizu *et al*., 1990). In the same model, CYP2E1 protein, but not its encoding RNA was induced in the kidney, brain and intestine as well as the liver, with a rapid decline after

removal of ethanol (Roberts *et al.*, 1994). Ethanol given in the drinking-water to rats induced CYP2E1 protein and nitrosodimethylamine (NDMA) demethylase activity in the brain, especially in neuronal cells in several regions (Anandatheerthavarada *et al.*, 1993). CYP2E1 protein induction by inhaled ethanol was demonstrated in Wistar rats in the centrilobular region of the liver, in alveolar cells of the lung and in proximal convoluted kidney tubules (Zerilli *et al.*, 1995). Ethanol at 5% in liquid diet (LDC-type) caused a marked increase in CYP2E1 and CYP1A2 protein and a small increase in CYP2B protein in rat lung, together with increased metabolism of the tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Ardies *et al.*, 1996). Ethanol in an LDC diet induced a 3.6-fold increase in CYP2E1 in rat pancreas (Kessova *et al.*, 1998). Induction of CYP2E1 was seen in peripheral blood lymphocytes of rabbits that received ethanol (10 or 15%) in the drinking-water for up to 24 days (Raucy *et al.*, 1995).

Enhancement of the activation of pro-carcinogens by treatment with ethanol was observed in several earlier experiments, and is presumed to be due to the induction of CYP2E1 in target tissues, although this induction was not demonstrated directly. In rats fed ethanol in an LDC diet, significantly enhanced capacity for the activation of *N*-nitrosopyrrolidine to a mutagen was observed in tissue extracts of the lung, liver and oesophagus but not the stomach: in this study mutagenicity was determined in a bacterial mutation assay with *Salmonella typhimurium* strain TA1535 (Farinati *et al.*, 1985). Treatment of rats with ethanol in an LDC-type liquid diet caused increased metabolism of inhaled benzene by hepatic microsomes, resulting in more rapid clearance of this compound *in vivo*. The treatment also enhanced the haemotoxicity of benzene, as was evident from a marked decrease in the number of peripheral white blood cell cells (Nakajima *et al.*, 1985). In C57Bl/6J mice, administration of 5 or 15% ethanol in the drinking-water for 13 weeks resulted in an enhancement of the toxic effects of inhaled benzene in the bone marrow, spleen and peripheral blood cells (Baarson *et al.*, 1982).

Recently, the role of CYP2E1 in the toxicity of xenobiotics was demonstrated more directly in *Cyp2e1*-deficient mice: azoxymethane caused fewer DNA adducts and colonic aberrant crypt foci compared with controls, consistent with the need for CYP2E1 to activate azoxymethane to the proximal carcinogen, methylazoxymethanol. The latter metabolite, however, was more active in *Cyp2e1*-deficient mice compared with controls; it was postulated that the lack of hepatic clearance resulted in greater dose delivery to the colon. In view of the very low level of CYP2E1 in the colon, the methylazoxymethanol produced from azoxymethane in the livers of normal mice would be transported to the colon, where it could damage DNA and initiate neoplasms (Sohn *et al.*, 2001).

(d) Effects of ethanol on expression of other CYPs

Ethanol in an LDC diet induced not only CYP2E1 (fivefold increase) in rat liver, but also CYP1A1, -2B1 and -3A (two- to fourfold); the latter activities persisted for several

days after withdrawal of ethanol (Roberts *et al.*, 1995b). Induction of CYP3A by chronic feeding of ethanol was confirmed in rat liver and hepatocytes by immunoblot analysis and by assessment of metabolism of fentanyl, a specific CYP3A substrate (Feierman *et al.*, 2003). Repeated acute oral treatment of rats with ethanol resulted in induction of CYP2B1 in the liver, but not in the brain (Schoedel *et al.*, 2001). Isopentanol, which is also present in alcoholic beverages, was a weak inducer of rat liver CYP2B and CYP3A when given in a liquid LDC diet, but synergized with ethanol to further increase the levels of these CYPs (Louis *et al.*, 1994).

High doses of ethanol administered to rats by the total enteric nutrition (TEN) method suppressed *Cyp3a2* mRNA and testosterone 6β-hydroxylation, but induced CYP3A9 in the liver; the latter, but not the former, effect was modulated by the fat/carbohydrate ratio of the liquid diet (Rowlands *et al.*, 2000). In the same model, CYP2C11 was suppressed in male rat liver and kidney, concomitant with a reduction in the amount and phosphorylation of the transcription factor STAT5b (Badger *et al.*, 2003). CYP2C11 is a growth hormone-regulated, male-specific steroid hydroxylase that may be involved in xenobiotic activation (Ozawa *et al.*, 2000). CYP2C7 and CYP2E1 were induced by ethanol in the colonic epithelium of rats (Hakkak *et al.*, 1996). Xenobiotics that are substrates for these members of the CYP2 and CYP3 families may be affected by such ethanol-induced changes.

(e) Effects of ethanol through alterations in detoxification

A single oral dose of ethanol given to rats enhanced the hepatotoxicity of 1,2-dibromoethane (IARC, 1999), a soil fumigant and animal carcinogen, due to ADH-dependent suppression of GST activity (Aragno *et al.*, 1996). In contrast, chronic treatment of rats with a diet containing ethanol led to small but significant increases in GST in the oesophagus (Farinati *et al.*, 1989).

(f) Effects of inhibition of CYPs by ethanol

Direct inhibition of CYPs by ethanol in peripheral target tissues may prevent metabolic activation of xenobiotics and hence reduce local toxic and tumorigenic effects. In contrast, inhibition of CYPs, especially CYP2E1, in the liver may reduce the clearance rate of CYP2E1 substrates and result in increased dose delivery to peripheral targets (reviewed in Anderson, 1992; Anderson *et al.*, 1995; Chhabra *et al.*, 1996). In early examples of this effect, intragastric administration of NDMA, a CYP2E1 substrate, in an alcoholic solution twice weekly to C57BL mice resulted in the development of olfactory neuroblastomas in 36% of the mice; this type of tumour was not seen with ethanol or NDMA alone. The percentage of mice with malignant liver tumours was reduced by the NDMA–ethanol treatment, which possibly reflects reduced NDMA activation and ensuing DNA damage in the liver (Griciute *et al.*, 1981). Ethanol as a solvent also enhanced the ability of NDEA and *N*-nitrosodipropylamine (NDPA) to cause malignant forestomach tumours and of NDPA to initiate lung tumours in C57BL mice

(Griciute *et al.*, 1982, 1987). However, the frequency of lymphomas induced by NDEA was significantly reduced when ethanol was used as a solvent (Griciute *et al.*, 1987). When dissolved in ethanol, NNN reduced the latency and increased the aggressiveness of olfactory tumours in BDVI rats (Griciute *et al.*, 1986). Because of the multiple-dose protocol in these experiments, several mechanisms for the effect of ethanol were possible, including altered disposition of the carcinogen within the organs, induction of CYP2E1 or other activating enzymes in the target tissue and/or tumour promotion.

The first hypothesis that ethanol influences the risk for nitrosamine-induced carcinogenesis through alterations in disposition resulted from a study by Swann *et al.* (1984). After acute administration, NDMA induced DNA-adduct formation in rat kidney only when given with ethanol, and ethanol increased alkylation of oesophageal DNA by NDEA. Inhibition of NDMA metabolism by liver slices from ethanol-treated Wistar-derived rats was demonstrated. In a later study in Fischer 344 rats, acute administration of ethanol (up to 20% v/v) by gavage with NMBzA resulted in increased DNA-adduct formation by the nitrosamine in the oesophagus (threefold), lung (twofold) and nasal mucosa (eightfold) (Yamada *et al.*, 1992). Various alcoholic beverages that are associated with risk for human cancer had similar or greater effects.

The interaction of ethanol with the metabolism and disposition of nitrosamines as illustrated above has been further studied in mice and monkeys, and showed effects of considerable magnitude. At concentrations of ~1 mM, ethanol completely inhibits the hepatic metabolism of NDMA *in vivo*, in hepatocytes and in hepatic microsomes (Tomera *et al.*, 1984; Anderson *et al.*, 1992a). The pharmacokinetic effects were studied in detail in mice (Anderson *et al.*, 1994) and patas monkeys (Anderson *et al.*, 1992b) *in vivo*. In mice given 0.5 mg/kg NDMA orally, pharmacokinetic parameters including clearance rate, residence times and AUC values, were increased 30-fold and 450-fold by simultaneous doses of 0.08 and 0.8 g/kg ethanol, respectively. In monkeys, 1.2 g/kg ethanol given orally before a 1-mg/kg intravenous dose of NDMA inhibited the clearance of the nitrosamine completely during 6 h, increased the mean residence time in the blood by about fourfold and the AUC by an average of 10-fold.

The effects of ethanol on NDMA clearance were associated with marked enhancement of toxic effects in peripheral tissues. Strain A mice treated with NDMA at several doses in the presence of 10 or 20% ethanol in the drinking-water for 12 weeks developed a greater number of lung tumours than mice given NDMA only (Anderson, 1988). Increased numbers of kidney tumours were also noted (Anderson *et al.*, 1992a). Similar results were obtained in these mice with a single intragastric dose of 5 mg/kg NDMA; inclusion of ethanol with the NDMA caused a dose-dependent increase in the incidence of lung tumours, with a ninefold enhancement at 20% ethanol (Anderson, 1992). This single-dose experimental design made it less likely that the effects of ethanol were due to the induction of CYP2E1 in the lung or to tumour promotion. Such effects were also ruled out by the observation that 10% ethanol in the drinking-water had no effect on the lung tumorigenicity of NDMA given by other routes: ethanol had to be delivered to the liver as a bolus with NDMA to have a significant effect. Mechanistic

relationships were further confirmed by the observation that the effects of ethanol on the NDMA AUC, on the O^6-methylguanine–DNA adducts levels in the lung and on the average numbers of lung tumours were of the same magnitude (Anderson *et al.*, 1992a).

Similar effects were seen with 6.8 ppm NDEA in strain A mice; inclusion of 10% ethanol in the drinking-water resulted in a fourfold increase in multiplicity of lung tumours and a 16-fold enhancement of the incidence of forestomach tumours. Inclusion of 10% ethanol with 40 ppm NPYR resulted in a 5.5-fold increase in lung tumour multiplicity (Anderson *et al.*, 1993).

In patas monkeys, the toxic and possibly pre-tumorigenic effects of NDMA were studied by use of O^6-methylguanine–DNA adducts as markers after an oral dose of 0.1 mg/kg NDMA, with or without a preceding dose of 1.6 g/kg ethanol (Anderson *et al.*, 1996). These DNA adducts were detected in all tissues, and were increased by co-exposure to ethanol in all tissues except the liver. Particularly striking effects were seen in the oesophagus (17-fold increase), colonic mucosa (12-fold), pancreas (sixfold), urinary bladder (11-fold), ovary (ninefold), uterus (eightfold), brain (ninefold) and spleen (13-fold). The large increase in DNA adducts in the oesophagus and in other peripheral organs as a result of the suppression of clearance of carcinogens may provide a mechanistic explanation for the enhancement of the risk for cancer from smoking by alcoholic beverage consumption (Tuyns, 2001).

The modulating effect of ethanol on nitrosamine clearance has also been studied in reproductive and perinatal studies. In a study with Sprague-Dawley rats, 1.6 g/kg ethanol was given by gavage to nursing dams followed by 5 mg/kg NDMA or 50 mg/kg NNK (Chhabra *et al.*, 2000). Ethanol resulted in a 10-fold increase in O^6-methylguanine–DNA adducts in maternal mammary glands after administration of NDMA and a smaller but significant increase in adduct levels after administration of NNK. Adducts in maternal blood cells also increased. In the suckling infants, DNA adducts were detected in the lungs and kidneys after maternal exposure to NDMA. The adduct levels increased about fourfold after maternal co-treatment with ethanol; maternal exposure to NNK did not result in DNA adducts in the infant tissues. In rats, NNK is not metabolized by CYP2E1 but rather by CYP1A2, -2A3, -2B1 and -2C6 (Jalas *et al.*, 2005). The effects of ethanol on NNK-derived DNA adducts in the maternal tissues suggests that inhibition by ethanol of one or more of these CYP isoforms could impact NNK clearance.

In pregnant patas monkeys, 1.6 g/kg ethanol given orally before an intragastric dose of 1 mg/kg NDMA resulted in a 50% reduction in O^6-methylguanine–DNA adducts in placenta and fetal liver, where adducts were relatively high. In contrast, a 1.5–2.5-fold increase in these adducts was observed in 11 other fetal tissues (Chhabra *et al.*, 1995). These results are consistent with the blockage of both metabolic activation in and clearance of NDMA from placenta and fetal liver by ethanol, which results in increased dose delivery to downstream target organs.

Inhibition of the clearance of carcinogens as a mechanism by which ethanol enhances carcinogenesis by these chemicals leads to the prediction that the enhancing

effects should not be seen if animals are treated with the same concentrations of ethanol and chemical carcinogen, but at different times and/or by different routes, which minimizes co-exposure. Several studies have confirmed this hypothesis. When NDEA was given to rats orally five times a week, followed each day by 25% ethanol (5 mL/rat/day), enhancement of oesophageal carcinogenesis in rats was not observed (Habs & Schmähl, 1981). In contrast, chronic exposure to ethanol in a liquid diet, which ensures constant and persistent concentrations in the blood, increased the incidence of nasal cavity and tracheal tumours in hamsters given NPYR intraperitoneally (McCoy *et al.*, 1981); however, when ethanol was given in the drinking-water (which would have provided primarily nocturnal exposure) no effect was seen on the incidence of tracheal tumours (McCoy *et al.*, 1986). Inclusion of ethanol in a liquid diet also led to an increased incidence of nasal cavity tumours in rats when NNN was co-administered in the liquid diet, but not when the carcinogen was given subcutaneously (Castonguay *et al.*, 1984). Ethanol in the drinking-water at 10% or given intrapharyngeally as a 50% solution did not alter the incidence of rat oesophageal tumours induced by *N*-nitrosopiperidine in the diet (Konishi *et al.*, 1986). In mice, 10% ethanol given with NDMA in the drinking-water resulted in a fivefold increase in the number of lung tumours, but had no significant effect on these numbers when NDMA was given by other routes (intragastrically, intraperitoneally, subcutaneously or intravenously) (Anderson *et al.*, 1992a). These findings support the hypothesis that direct inhibition of carcinogen clearance by ethanol is the operative mechanism. It is unlikely that hormonal change, tumour promotion or various cellular alterations give rise to the effects of ethanol. Alcohol-mediated facilitation of cellular penetration by the carcinogens remains a possible alternative.

Finally, if inhibition of CYP2E1 is responsible for the enhancement of the effects of these various nitrosamines by ethanol, then other CYP2E1 inhibitors should have a similar effect. This has indeed been shown for the CYP2E1 inhibitor disulfiram, which caused an increase in the incidence of paranasal sinus tumours after administration of NDMA, and of oesophageal tumours after administration of NDEA to rats (Schmähl *et al.*, 1976).

This toxicokinetic-based enhancement of genotoxic and tumorigenic effects, which is seen so clearly for nitrosamines, does not necessarily apply consistently to other substrates of CYP2E1. Urethane is activated and metabolized by CYP2E1 (Hoffler & Ghanayem, 2005; Ghanayem, 2007) and this metabolism is inhibited by ethanol (Waddell *et al.*, 1987; Yamamoto *et al.*, 1988; Carlson, 1994; National Toxicology Program, 2004). However, the effects of ethanol on urethane carcinogenicity have been mixed. In a chronic administration model, 10 or 20% ethanol given to A/Ph female mice in the drinking-water together with 200, 500 or 1000 ppm urethane resulted in a reduced multiplicity of lung tumours (Kristiansen *et al.*, 1990).

In B6C3F1 mice, 5% ethanol given with 10, 30 or 90 ppm urethane decreased the incidence of lung tumours in males, whereas 5% ethanol with 10 ppm urethane increased the incidence of these tumours in females. The incidence of Harderian gland

tumours was also decreased by ethanol in males, but only at the 30-ppm urethane dose, and that of haemangiosarcomas of the heart was increased in females, but only at the 90-ppm urethane dose. Interpretation of these results is somewhat hindered by effects of the chemicals on body weights (National Toxicology Program, 2004).

In contrast to low-molecular-weight nitrosamines, which are completely degraded in the liver, urethane is metabolized to an epoxide as proximate carcinogen, with sufficient stability to be carried from the liver to downstream targets (Park *et al.*, 1993). This may explain the reduced carcinogenicity of urethane plus ethanol in some situations.

4.4.3 *Comparison of humans and animals*

(a) Ethanol

Most studies of ethanol metabolism in experimental animals have employed rats, which appear to be a reasonably good model for humans. A few comparative studies have included both species. Localization of ethanol-induced CYP2E1 in the liver (Tsutsumi *et al.*, 1989) and the effect of concentration of ingested ethanol on its pharmacokinetics (Roine *et al.*, 1991) were similar in humans and rats. There is evidence from both humans and rats that chronic exposure to high levels of ethanol, with damage to the gastric mucosa, results in a reduction in gastric ADH (see earlier sections). There have been varying conclusions about the relative importance of gastric versus hepatic first-pass clearance of ethanol for both humans and animals. According to recent physiologically based pharmacokinetic modelling data, gastric metabolism may play a greater role in rats than in humans. In rats, gastric ADH is the high-K_m class IV isoform, ADH7. In the human stomach, three isoforms may be represented from classes I, II and IV, but again ADH7 accounts for most of the activity. Human and rat ADH7 are 88% homologous, but affinities of human and rat ADH7 for ethanol are markedly different: the K_m is 2.4 M for rats and 37 mM for humans (Farrés *et al.*, 1994b). This difference is consistent with greater first-pass metabolism of ethanol in the rat versus the human stomach.

Levels of ADH activity (V_{max}) were found to be about sixfold lower in human than in rat liver (Sinclair *et al.*, 1990) and varied with body weight, as is usual for metabolic parameters (Matsumoto *et al.*, 1999). Possibly as a consequence of this slower ethanol degradation by ADH, the in-vivo induction in the liver of the gene encoding CYP2E1 may occur at lower concentrations of ethanol in humans than in rats. In the latter, blood concentrations >3 g/L were required to increase hepatic *CYP2E1* mRNA (Badger *et al.*, 1993), whereas the alcohol drinkers who showed a marked increase in hepatic *CYP2E1* mRNA in the study of Takahashi *et al.* (1993) must have had lower levels of blood ethanol. Ethanol and isopentanol were more effective in inducing CYP3A in human than rat hepatocytes in culture (Kostrubsky *et al.*, 1995). As noted above, primary hepatocytes from humans, but not from rats, responded to ethanol with an increase in *CYP2E1* mRNA. These results together suggest that the interaction of ethanol with CYPs is more prominent and important in humans than in rats.

(b) Xenobiotics

Both the inductive and the inhibitory effects of ethanol on several CYPs that act on xenobiotics have been observed in humans and animals, although the human data are limited in scope. The marked effects of ethanol on induction of pro-mutagenic DNA adducts by NDMA in a non-human primate (Anderson *et al.*, 1996) indicate that the relationships between inhibition of hepatic clearance of NDMA (and other nitrosamines) by ethanol and the induction of DNA adducts and tumours in extrahepatic targets, which are seen so clearly in rodents, may also pertain to humans. The magnitude of these effects in rodents has often been large (commonly five- to 10-fold), and greater than the tumour-enhancing effects of ethanol in other rodent-based mechanistic models. This comparison suggests that the toxicokinetic hypothesis should be considered to be important, especially in view of the tobacco–alcohol synergisms that are seen with respect to cancer incidence in smokers who consume alcohol.

(c) Interaction of ethanol and tobacco

The combined effects of alcoholic beverages and tobacco on cancer incidence and mortality have been widely studied in many populations. In the more recent studies on multiplicative and additive interactions, synergistic effects of alcoholic beverages and tobacco have been found, especially for oropharyngeal and oesophageal cancers (Castellsagué *et al.*, 2004; Lee *et al.*, 2005).

Although high alcoholic beverage consumption by itself may increase the risk for human head and neck cancers, the effect is much smaller than that of tobacco alone. It seems probable that the synergism between tobacco and alcoholic beverages in the causation of these cancers is due to the enhancement of the effects of tobacco carcinogens by ethanol.

There are data to support at least three possible mechanisms for the enhancing effects of alcoholic beverages on the risk for oropharyngeal and oesophageal cancer due to tobacco.

First, alcohol may have a local permeabilizing effect on penetration of the oral mucosa by tobacco carcinogens (Du *et al.*, 2000).

Additional possible mechanisms may involve CYP2E1 and other enzymes that both activate and detoxify carcinogens present in tobacco, including NDMA, NDEA, NNK, benzene and others. As noted above, ethanol induces CYP2E1 in all species tested, CYP3A4 and probably CYP1A2 in humans and CYP1A1, -2B1 and -3A in rat liver. In rats, ethanol in a liquid diet induced CYP2E1 in epithelia of the cheek, tongue, oesophagus and forestomach (Shimizu *et al.*, 1990); similar inductive events probably occur in humans. Treatment of rats with ethanol using this model resulted in an increased capacity of oesophageal tissue to activate NPYR to a mutagen (Farinati *et al.*, 1985). [The Working Group noted that the induction of CYP2E1 in this study was presumed but not actually measured.] Thus, the induction of CYPs that bring about the

metabolic activation of tobacco carcinogens in target tissues could explain part of the enhancing effects of alcoholic beverages.

A third mechanistic possibility for the enhancing effect of alcohol consumption on tobacco-related cancers arises from the fact that ethanol competitively inhibits hepatic metabolism by CYP2E1 in all species tested, as well as human CYP1A1, -2B6 and -2C19 (see previous sections). This inhibition could result in increased exposure of tissues other than liver and genotoxicity in those tissues induced by tobacco carcinogens that are substrates for these enzymes. Ethanol caused a nearly fivefold increase in oesophageal DNA adducts in rats treated with NDEA (Swann *et al.*, 1984). In monkeys treated with NDMA, alcohol caused a 17-fold increase in oesophageal DNA adducts and a fivefold increase in nasal cavity tissue adducts (Anderson *et al.*, 1996). In each of these studies, ethanol treatment was acute, so that enzyme induction was unlikely. Also, the oesophagus was not directly exposed to either ethanol or carcinogen, which indicates that a systemic interaction, presumably inhibition of hepatic carcinogen clearance, was responsible for the observed effects in the oesophagus and nasal cavity.

The relevance of these findings to tumorigenesis is confirmed by the results of several studies with experimental animals. Daily treatment of rats with NDEA in 30% ethanol caused more oesophageal papillomas than NDEA without ethanol (Gibel, 1967). Repeated oral dosing of mice with NDMA in 40% ethanol resulted in the appearance of nasal cavity tumours that were not seen with NDMA or ethanol alone (Griciute *et al.*, 1981). Inclusion of 10% ethanol in the drinking-water led to a fivefold increase in the incidence of oesophageal tumours in rats caused by NDEA (Aze *et al.*, 1993). Ethanol given in a liquid diet resulted in a significant increase in the incidence of nasal cavity and tracheal tumours in hamsters caused by intraperitoneal injection of NPYR (McCoy *et al.*, 1981). In these studies, CYP enzyme induction was possible, as well as tumour promotion and other effects of the chronic administration of ethanol, but, in view of the marked effects of acute exposures on DNA adducts, inhibition of carcinogen clearance by ethanol may be the best supported interpretation at present.

4.5 Major toxic effects

4.5.1 *Humans*

(a) Alcohol

(i) *Liver*

Chronic ethanol ingestion results in steatosis, steatohepatitis, fibrosis and cirrhosis of the liver. The risk for cirrhosis increases with daily alcoholic beverage intake of >60–80 g per day in men and >20 g per day in women (reviewed in Mandayam *et al.*, 2004). Dose-dependent increases in risk for alcoholic liver disease are observed in both genders (Becker *et al.*, 1996a). Hispanics and blacks have higher cirrhosis-related mortality rates than non-Hispanic whites in the USA, but it is unclear whether the differences

are attributable to genetic differences or are influenced by lifestyle or socioeconomic status (reviewed in Mandayam *et al.*, 2004). The super-active *ADH1B*2* allele and the inactive *ALDH2*2* allele are preventive factors against alcoholism (Harada *et al.*, 1985; Mulligan *et al.*, 2003). These alleles are less frequent in patients with alcoholic liver disease than in general populations (Chao *et al.*, 1994; Tanaka *et al.*, 1996). However, a recent review and a meta-analysis have shown that polymorphisms of genes encoding alcohol-metabolizing enzymes (ADH1B, ADH1C, ALDH2 and CYP2E1) are unlikely to make a significant contribution to the development of alcoholic liver disease among drinkers who consumed the same amounts of alcoholic beverages (reviewed in Stickel & Österreicher, 2006; Zintzaras *et al.*, 2006). Alcoholics are frequently infected HCV (10% in the USA, 14% in Europe, 45–80% in Japan), and numerous studies have found that alcoholic beverage consumption is detrimental to HCV patients (reviewed in Jamal *et al.*, 2005). Alcohol and HCV infection independently increase the risk for HCC, and there may be synergism between the two factors, with HCC occurring at an earlier age and being more advanced in patients who consume alcohol (reviewed in Morgan *et al.*, 2004).

The interaction between alcoholic beverages and HBV is not completely understood. Several studies have reported a positive interaction, but others have shown negative results (reviewed in Mandayam *et al.*, 2004).

(ii) *Pancreas*

Acute and chronic pancreatitis is a well documented alcohol-related disease. Excessive alcohol use accounts for 70–90% of chronic pancreatitis in western countries (Gullo, 2005). The risk for chronic pancreatitis increases in proportion to dose and duration of alcoholic beverage consumption. Ethanol is metabolized in the pancreas to produce toxic metabolites such as acetaldehydes and FAEEs. According to the estimate by Apte and Wilson (2003), the average alcoholic beverage consumption in patients who develop chronic pancreatitis is 150 g ethanol per day for a period of 10–15 years. Alcoholic pancreatitis begins as an acute process and progresses to a chronic condition with recurrent episodes of acute attack, which show endocrine and exocrine dysfunction (*diabetes mellitus* and steatorrhoea). Tobacco smoking and a diet rich in protein and fat are suspected to be contributing factors (Gullo, 2005). The histopathological features of alcoholic pancreatitis are reviewed in more detail elsewhere (Apte & Wilson, 2003; Gullo, 2005).

While moderate alcoholic beverage consumption has generally been related to a decreased risk for type-2 *diabetes mellitus* (Koppes *et al.*, 2005), high alcoholic beverage consumption was associated with an increased risk for this disease (Tsumura *et al.*, 1999) and for glucose intolerance (Sakai *et al.*, 2006) in Japanese, who may have a lower capacity for insulin secretion than Caucasians (Fukushima *et al.*, 2004).

(iii) *Gastrointestinal tract*

Tissue-specific alcohol metabolism

Ethanol concentrations in the colonic lumen as well as in saliva are similar to blood levels in the post-distribution phase (15–120 min after an ethanol challenge), and ethanol in the saliva and colonic lumen is largely derived from the blood stream (Halsted *et al.*, 1973; Salaspuro, 1996). Microbial oxidation of ethanol contributes to the majority of acetaldehyde formation in the saliva and colonic contents. Fairly high levels of acetaldehyde have been measured in human saliva after a moderate dose of ethanol (0.5 g/kg bw). The production of acetaldehyde was reduced after antiseptic mouth rinsing (Homann *et al.*, 1997). Acetaldehyde levels in saliva after ethanol intake were nine times higher in individuals with partially defective ALDH2 than in those with normal activity of this enzyme, but the in-vitro capacity of saliva to produce acetaldehyde from ethanol was the same in both groups. It was concluded that acetaldehyde is also produced in the salivary glands (Väkeväinen *et al.*, 2000).

Histopathology

Ethanol causes a diversity of morphological and functional alterations along the gastrointestinal tract, which differ somewhat in different segments (Siegmund *et al.*, 2003; Rajendram & Preedy, 2005). The consumption of strong alcoholic beverages directly causes local mucosal injury in the oropharynx, oesophagus, stomach and upper part of small intestine (Simanowski *et al.*, 1995). A typical example is haemorrhagic erosion of the gastric and duodenal mucosa. Chronic administration of ethanol results in toxic damage to the gastrointestinal mucosa followed by epithelial regeneration. Hyperproliferation of epithelial cells is a histological feature that is typical of the regeneration process. Highly proliferative cells have a greater chance of DNA replication errors that result in genetic alterations (Simanowski *et al.*, 1995). The toxic effects of ethanol in the upper gastrointestinal tract may be ascribed in part to acetaldehyde that is generated through oxidation of ethanol in the saliva, as is the case in the large intestine where acetaldehyde is mostly generated by colonic microbes (Salaspuro, 2003).

In a comparative study of alcoholics with a mean intake of >100 g ethanol per day and non-alcoholics with a mean intake of <30 g ethanol per day (Simanowski *et al.*, 2001), increased rectal cell proliferation, as determined by histochemical staining, was reported among the alcoholics. The investigators also noted expansion of the proliferative compartment in the rectal mucosa. Alcohol-related histological and molecular changes in the gastrointestinal tract are summarized in detail elsewhere (Simanowski *et al.*, 1995; Siegmund *et al.*, 2003; Rajendram & Preedy, 2005).

Other pathophysiological effects

Sparse literature concerning humans indicates that alcoholic beverage consumption is related to decreased cellular immunity in the small intestine (MacGregor, 1986; Rajendram & Preedy, 2005). Malabsorption of macronutrients and micronutrients

and inadequate dietary intake are known to occur in alcoholics (Bode & Bode, 2003; Manari *et al.*, 2003), and folate is one of the most common nutrients that are deficient. Chronic alcoholic beverage consumption is associated with reduced absorption of water and sodium in the jejunum and ileum, which gives rise to the diarrhoea seen among alcoholics (reviewed in Bode & Bode, 2003).

(iv) *Endocrine organs*

Ethanol affects the function of endocrine organs such as the gonads, anterior and posterior pituitary glands, pancreas, thyroid and adrenal glands (reviewed by Adler, 1992). Some studies also suggest that ethanol may affect gonadotropin secretion at the hypothalamus and/or anterior pituitary (Iranmanesh *et al.*, 1988). The effects of ethanol on sex hormones are of particular interest with regard to the potential mechanism of breast cancer.

Effects on sex hormones in women

In women, chronic consumption of alcoholic beverages may result in estrogen deficiency, anovulation and amenorrhea (Mendelson & Mello, 1988). In particular, alcoholic beverage intake in very large amounts has been associated with menstrual cycle irregularities, anovulation and early menopause (Hugues *et al.*, 1980). However, for moderate alcohol consumption, there is growing evidence of a positive association with the sex hormones that are linked to breast cancer (i.e. estradiol, dehydroepiandrosterone, androstenedione and testosterone).

Many observational studies on ethanol consumption and serum hormone levels were limited by small sample sizes and/or limited ranges of alcoholic beverage intake. In the largest cross-sectional study reported to date, serum samples collected from 790 pre- and 1291 postmenopausal women in eight European countries who were not taking exogenous hormones were assessed for endogenous sex steroids and sex hormone-binding globulin (SHBG) concentrations (Rinaldi *et al.*, 2006). Premenopausal women who consumed more than 25 g alcohol per day had nearly 40% higher estrone, 20% higher androstenedione and 30% higher dehydroepiandrosterone sulfate, testosterone and free testosterone concentrations compared with women who were non-drinkers, while SHBG concentrations showed no association with alcoholic beverage intake. In postmenopausal women, the serum concentrations of all steroids mentioned above were 10–20% higher in women who consumed more than 25 g alcohol per day compared with non-drinkers, while SHBG levels were about 15% lower. Estradiol or free estradiol did not show any association with alcoholic beverage intake in either pre- or postmenopausal women.

In controlled feeding studies with human volunteers, a direct relationship was found between alcoholic beverage intake and circulating androgen and estrogen levels (Reichman *et al.*, 1993; Ginsburg *et al.*, 1996; Sarkola *et al.*, 1999, 2000, 2001; Mahabir *et al.*, 2004; Sierksma *et al.*, 2004). In a study of postmenopausal women who were not taking hormone replacement therapy, and who consumed either 15 or 30 g alcohol per day in a controlled diet for 8 weeks, serum concentrations of estrone

sulfate significantly increased by 7.5% and 10.7%, and dehydroepiandrosterone sulfate increased by 5.1% and 7.5%, respectively, relative to the concentrations measured in women who consumed placebo. In this study, there was no change in estradiol, testosterone or progesterone levels (Dorgan *et al.*, 2001). In a cross-sectional study of premenopausal women who were not taking oral contraceptives, alcohol ingestion was not associated with plasma estrogen concentrations at any of three time intervals during the menstrual cycle. Alcohol consumption was positively associated with average plasma concentrations of androstenedione (Dorgan *et al.*, 1994).

A study in premenopausal women (mean age, 23–32 years) showed that acute intake of alcohol (0.7 g/kg) induced a significant increase in plasma estradiol levels, which reached a peak value at 25 min after initiation of drinking when blood alcohol levels averaged 34 mg/mL (Mendelson *et al.*, 1988). In premenopausal women (aged ~25–35 years), ethanol was found to elevate testosterone levels in blood plasma regardless of the dose of alcohol (0.3–1.0 g/kg). This effect was most pronounced during the ovulatory phase of the normal menstrual cycle and in women who were currently using oral contraceptives (Eriksson *et al.*, 1994), and has been attributed to inhibited catabolism of testosterone in the liver (Sarkola *et al.*, 2001).

Observational and intervention studies generally suggest that alcoholic beverage intake is associated with increased levels of estradiol in plasma. These findings led to the hypothesis that the elevation of estradiol plays a role in the mechanism that underlies the association between alcoholic beverage consumption and the development of breast cancer (Pöschl & Seitz, 2004).

The mechanism by which ethanol affects the levels of sex hormones in women has been suggested to be an ethanol-mediated increase in the liver redox state, which is represented by an increase in the hepatic NADH-to-NAD ratio that decreases steroid catabolism (Sarkola *et al.*, 1999, 2001). Alternatively, it has been hypothesized that the effect of alcoholic beverage intake, even of moderate amounts, on circulating sex hormone concentrations may be mediated by melatonin, which inhibits estrogen production (Stevens *et al.*, 2000). In addition, some alcoholic beverages contain phytoestrogens that may contribute to total estrogen in plasma (Gavaler, 1998).

Effect on sex hormones in men

Studies in alcoholic men showed that ethanol and its metabolites have direct toxic effects on the testes, which results in decreased testosterone levels and reduced sexual function (IARC, 1988). Among non-alcoholic men, a high dose of alcohol (>1 g/kg) has been found to decrease the concentration of circulating testosterone (Välimäki *et al.*, 1984, 1990). The effect is more pronounced at the later stage of intoxication and during the hangover phase, which has been attributed to a physiological stress condition associated with elevated cortisol levels (Välimäki *et al.*, 1984). The reduction in testosterone has generally been explained, on the basis of research in experimental animals, by direct inhibition of testosterone biosynthesis in the testis (Eriksson *et al.*, 1983). In contrast to high doses of alcohol, lower doses seem to elevate testosterone levels in men (Sarkola & Eriksson, 2003). It is not clear under what conditions this effect occurs.

(v) *Cardiovascular system*

Alcoholic beverage consumption poses a substantial risk for cardiovascular diseases overall, but a J-shaped curve has been noted for light-to-moderate drinking, which is associated with a protective effect on the cardiovascular system.

The mechanism of the protective effect of moderate alcohol intake was explained by the dose-dependent ability of ethanol to increase high-density lipoprotein cholesterol, decrease low-density lipoprotein cholesterol, reduce plasma fibrinogen, inhibit platelet aggregation and reduce plasma apolipoprotein (A) concentration. Thus, ethanol at moderate doses reduces the risk for cardiovascular diseases by inhibiting the formation of atheroma and by decreasing the rate of blood coagulation (Agarwal, 2002; Klatsky, 2002).

Various mechanisms have been suggested for ethanol-mediated cardiovascular pathologies. FAEEs, esterification products of fatty acids and ethanol are mediators of ethanol-induced cell injury (Laposata *et al.*, 2002). Chronic ethanol-induced damage to the vascular endothelium has been linked to the increased release of tumour necrosis factor α (Luedemann *et al.*, 2005). Apoptosis is implicated in the pathogenesis of ethanol-induced tissue damage including that of the cardiac muscle (Fernández-Solà *et al.*, 2006).

The role of heavy drinking in the development of cardiac disease has been observed in humans as well as in various animals species. Abnormalities include reduction of ventricular function, and metabolic and morphological changes. Increased cardiovascular risks of heavy drinking include various effects, such as alcoholic cardiomyopathy, hypertension, arrhythmia and a haemorrhagic stroke (Regan *et al.*, 1977).

A recent meta-analysis summarized the findings on the association between alcoholic beverage consumption and the risk for stroke (Reynolds *et al.*, 2003). From 122 studies, a random-effects model and meta-regression analysis were used to obtain the overall results. Compared with abstaining, heavy drinking of more than 60 g alcohol per day was associated with an increased relative risk for total stroke, ischaemic stroke and haemorrhagic stroke (relative risk range, 1.64–2.18), while drinking of less than 12 g alcohol per day was associated with a reduced risk for total stroke and ischaemic stroke (relative risk, 0.83 and 0.80, respectively) and drinking of 12–24 g per day with a reduced relative risk for ischaemic stroke (relative risk, 0.72). The analysis supported a significant non-linear relationship of alcoholic beverage consumption with total and ischaemic stroke, and a linear relationship with haemorrhagic stroke.

The association between alcoholic beverage consumption and the risk for coronary heart disease has been reviewed (Marmot, 1984, 2001). Based on seven longitudinal studies and six case–control studies, an increased risk among heavy drinkers and a reduced risk among moderate drinkers were found. Other reviews or meta-analyses generally corroborated these findings (Rimm *et al.*, 1996; Corrao *et al.*, 2000). Evidence from eastern Europe showed that irregular (binge) drinking caused cardiovascular disease even at the level of moderate alcohol intake (Britton & McKee, 2000).

Therefore, not only the amount but also the pattern of drinking is important in assessing the effects of alcoholic beverage consumption. Binge drinking may increase silent myocardial ischaemia in those with pre-existing coronary artery disease, marked fluctuation in blood pressure, adverse changes in the balance of fibrinolytic factors and ethanol-induced arrhythmia (Puddey *et al.*, 1999).

A recent position paper was published by the National Institute on Alcohol Abuse and Alcoholism on the health risks and potential benefits of moderate alcoholic beverage use (Gunzerath *et al.*, 2004). This paper concluded that consumption of two drinks per day for men and one for women is unlikely to increase health risks, and cautioned that men should not exceed four drinks on any day and women not exceed three on any day, with emphasis on the importance of drinking patterns as well as the amount consumed.

In contrast to numerous original studies and meta-analyses that support the J-shaped association between alcoholic beverage consumption and cardiovascular risk, a recent meta-analysis argued that the apparent cardioprotective effect of moderate drinking arose from a misclassification bias by including in the category 'abstainers' those who had reduced or stopped drinking in view of their age or ill health (Fillmore *et al.*, 2006).

(vi) *Immune system*

The adverse effects of ethanol on the host defence system have been known for a long time, based on the observations that alcoholics are vulnerable to various infectious agents. In addition, once certain types of infection occur, the course tends to be more severe, with higher rates of complications and mortality (Brayton *et al.*, 1970). Carefully controlled studies have been conducted to avoid confounding by nutritional deficiency and complications from alcoholic liver diseases. Findings from clinical and experimental studies have been summarized in several recent reviews (Szabo, 1999; Díaz *et al.*, 2002; Pavia *et al.*, 2004). The effects of ethanol on immunity are widespread over many aspects of the immune system. The immune system functions in two main components: innate, or non-specific, immunity and adaptive, or specific, immunity. The innate immune system involves mainly macrophages and neutrophils that provide a first line of defence. The adaptive immune system involves lymphocytes such as T cells and B cells, and responds to the specific antigens that escape the defence by innate immunity. Numerous studies have shown that ethanol affects both innate and adaptive immune systems.

Inflammation is a key aspect of innate immunity in response to bacterial pathogens. Macrophages and neutrophils play major roles in the inflammatory process to destroy pathogens, and various cytokines are secreted to maintain communication among cells. Exposure to ethanol impairs phagocytic function of macrophages and neutrophils, as observed in human and animal studies. In chronic alcoholic beverage abusers, inflammatory cytokine levels were significantly increased, leading to the pathological changes observed in alcoholic hepatitis (Szabo, 1997, 1999).

The most important cells involved in the adaptive immune system are T and B lymphocytes. Both groups of cell are affected by chronic exposure to ethanol. The numbers of all subpopulations of T cells are decreased in humans and animals during chronic ingestion of ethanol. Ethanol reduces the ability of T cells to proliferate appropriately in response to an antigen. Acute exposure to ethanol induced programmed cell death or apoptosis of T cells. Overall, exposure to ethanol resulted in a reduced cell-mediated immune response that depended on T cells (Szabo, 1999). The effects of ethanol on B cells mainly appeared to be the elevated levels of serum antibodies (Cook, 1998). Total serum immunoglobulin E (IgE) is increased by alcoholic beverage intake, and the causal role of ethanol seems well supported. The mechanism of this effect is not clear, and several possibilities have been suggested: a direct effect on B cells that increases IgE production, or an ethanol-induced increase in intestinal wall permeability which may result in increased exposure to antigens. Alterations in the cytokine balance that favour Th2 cytokine predominance may also promote IgE synthesis (Gonzalez-Quintela *et al.*, 2004).

The effects of ethanol on the immune response, particularly the stimulation of cytokine secretion, are known to result in tissue damage in alcoholic hepatitis patients (Martinez *et al.*, 1992). Associated with induction of CYP2E1, an altered immune response increases susceptibility to viral infection from HBV and HCV (Djordjević *et al.*, 1998; Albano, 2006). Furthermore, ethanol-induced immunosuppression was hypothesized to be a cofactor in the promotion of cancer in general (Mufti *et al.*, 1989).

Emerging evidence suggests that ethanol acts as a neurochemical messenger that affects the network of the nervous, endocrine and immune systems (Haddad, 2004). In particular, ethanol regulates the hypothalamus–pituitary–adrenal axis that modulates the release of hormones, especially adrenocorticotropic hormone and corticosterone, which in turn influences the immune status.

(b) Acetaldehyde

(i) *Irritation of the eyes and the respiratory tract*

Upon acute exposure to moderate concentrations of acetaldehyde, humans experience irritation of the eyes and respiratory tract. In a study with 24 volunteers, eye irritation occurred in sensitive persons after a 15-min exposure to a concentration of 25 ppm and, in the majority, after exposure to 50 ppm. Irritation of the respiratory tract was noted at around 130 ppm during 30 min, and irritation of nose and throat at 200 ppm during 15 min (Verschueren 1983). Intravenous infusion of young male volunteers with 5% (v/v) acetaldehyde at a rate of approximately 20–80 mg/min for up to 36 min resulted in an increased heart rate, increased ventilation rates and respiratory dead space, and a decreased alveolar carbon dioxide level (Asmussen *et al.* 1948). The irritant effects of acetaldehyde vapour, such as coughing and and a burning sensation in the nose, throat and eyes, usually prevents exposure to concentrations that are sufficient to cause depression of the central nervous system (IARC, 1985). The results

of one study in human volunteers indicated that acetaldehyde penetrates the human blood-cerebrospinal fluid barrier (Hillbom *et al.* 1981).

(ii) *Dermal effects*

Prolonged dermal exposure to acetaldehyde can cause erythema and burns in humans; repeated contact may result in dermatitis, due to irritation or sensitization (IARC, 1985). In patch tests on dry skin, acetaldehyde (10%) caused local cutaneous erythema in 12 volunteers (Haddock & Wilkin 1982). The ethnic predisposition to ethanol-provoked flushing among diverse East Asian populations is probably the consequence of accumulation of acetaldehyde. Topical application of acetaldehyde (75% in water) caused acute cutaneous erythema in 12 volunteers of Oriental ancestry. In persons with this genetic predisposition, cutaneous erythema was also observed after topical application of ethanol or propanol, and the cutaneous vascular reaction to these primary alcohols is probably provoked by the corresponding aldehyde (Wilkin & Fortner 1985a,b).

4.5.2 *Experimental systems*

(a) Ethanol

(i) *Liver*

A variety of mechanisms have been proposed to explain the pathogenesis of ethanol-induced liver injury (reviewed in Wheeler *et al.*, 2001a,b; Lieber, 2004b; Siegmund & Brenner, 2005; Albano, 2006; Dey & Cederbaum, 2006).

The pathological changes caused by alcohol in rodent liver are very similar to those observed in humans. Subchronic administration of alcohol to rats and mice leads to steatosis, steatohepatitis and initial stages of fibrosis. Cirrhosis has not been observed in rodent studies with alcohol alone. ADH-mediated ethanol metabolism modifies the cellular redox state (decreases the NAD^+/NADH redox ratio), which promotes steatosis by stimulating fatty acid synthesis and inhibiting fatty acid oxidation (reviewed in Lieber, 2004b). Administration of a bolus dose of ethanol to rats rapidly accelerated metabolism of ethanol in the liver of animals and resulted in downstream hypoxia in the pericentral region of the liver lobule (reviewed in Bradford & Rusyn, 2005). High doses of ethanol caused vasoconstriction and impaired microcirculation in isolated perfused rat liver (Oshita *et al.*, 1992). The development of hypoxia after acute administration of ethanol to rats could be confirmed by means of the hypoxia marker, pimonidazole (Arteel *et al.*, 1996).

An important enzyme in the microsomal ethanol-oxidizing system is the ethanol-inducible CYP2E1, which produces various reactive oxygen species, including the superoxide anion and hydrogen peroxide; more powerful oxidants, including the hydroxyl radical, ferryl oxidants and the 1-hydroxyethyl radical, are produced in the presence of iron (reviewed in Cederbaum, 2003). CYP2E1-derived oxidants stimulated type I collagen synthesis in hepatic stellate cells (the key cell type of liver fibrogenesis)

and caused mitochondrial injury and induction of oxidant damage to DNA in rodents (Bradford *et al.*, 2005; Albano, 2006). Polyenylphosphatidylcholine, a mixture of polyunsaturated phosphatidylcholines extracted from soya beans, decreased CYP2E1 activity in rats and inhibited hepatic oxidative stress and fibrosis in baboons fed ethanol (Lieber *et al.*, 1994). While ethanol-induced liver pathology correlated with CYP2E1 levels and increased lipid peroxidation in rats that had been intragastrically infused with ethanol (French *et al.*, 1993; Tsukamoto *et al.*, 1995), *CYP2E1*-knockout mice were not protected from ethanol-induced liver injury (Kono *et al.*, 1999).

Chronic feeding of ethanol decreased the number of microtubules (Matsuda *et al.*, 1979) and reduced the amount of tubulin in rat liver, which resulted in impaired microtubule-dependent protein trafficking and hepatocyte ballooning (Tuma *et al.*, 1991). Similar effects were seen with the oxidation products of ethanol, i.e. acetaldehyde and acetate. Decreased hepatic microtubules and increased hepatic export-protein content were observed in ballooned hepatocytes in patients with alcoholic liver disease (Matsuda *et al.*, 1985). The reactive compounds acetaldehyde, malondialdehyde, 4-hydroxy-2-nonenal and the 1-hydroxyethyl radical react with proteins to form protein adducts, which are immunogenic and may contribute to alcohol-induced liver tissue damage (reviewed in Albano, 2006).

Ethanol-induced oxidative stress causes dysfunction and depolarization of mitochondria and changes their permeability. These mitochondrial alterations are now recognized as a key step in apoptosis; they enhance the sensitivity of cells to other pro-apoptotic or damage signals (reviewed in Adachi & Ishii, 2002). The imbalance between oxidant production and hepatic antioxidant defence, especially by GSH, plays an important role in the pathogenesis of ethanol-induced liver injury. Reduction of mitochondrial GSH content by chronic administration of ethanol preferentially occured in pericentral hepatocytes (Hirano *et al.*, 1992). Introduction of the superoxide dismutase gene via adenovirus-mediated gene transfer (Wheeler *et al.*, 2001b) and the use of drugs or nutritional antioxidants, such as the GSH precursor *S*-adenosylmethionine, have been found to protect hepatocytes against ethanol-induced toxicity (reviewed in Lieber, 2002).

Ethanol-induced oxidative stress and induction of damage in mitochondrial DNA have been studied intensively in the liver of rodents, and these pathological processes are also conceivable in tissues other than the liver (Hoek *et al.*, 2002). Ethanol increases the generation of reactive oxygen species by enhanced redox pressure through NADH, which is produced during oxidation of ethanol by ADH (cytosolic NADH) and also upon oxidation of acetaldehyde by mitochondrial ALDH2. The induction of CYP2E1 by chronic heavy ethanol intake is a mechanism that explains the ethanol-induced increase in reactive oxygen species. Mitochondrial proteins and lipids as well as mitochondrial DNA are targets for oxidative damage. Damaged mitochondrial DNA results in mitochondrial dysfunction, and further increases the oxidative stress in the cell. Oxidative damage to mitochondrial DNA is inversely related to the lifespan of mammals (Barja & Herrero, 2000), and is purportedly linked to ageing (Raha & Robinson,

2000). Chronic administration of ethanol caused accumulation of damaged mitochondrial DNA and increased the amount of mitochondrial DNA strand breaks in the liver of rodents (Cahill *et al.*, 2002).

(ii) *Pancreas*

Both acute and chronic administration of high doses of ethanol resulted in a decrease in GSH, a reactive oxygen species scavenger, and an increase in oxidized GSH, proteins and lipids in the pancreatic tissue of rats (Altomare *et al.*, 1996; Grattagliano *et al.*, 1999). Other experiments in rats have shown a fivefold increase in CYP2E1 enzyme concentration in the pancreas and the induction of pancreatic hypoxia after chronic administration of ethanol (Norton *et al.*, 1998; McKim *et al.*, 2003). Chronic ethanol ingestion increased protein synthesis in the pancreas two- to threefold, as measured by the incorporation of ^{3}H-labelled leucine in rats *in vivo* after overnight fasting and *in vitro* in isolated pancreatic acini of these rats (Ponnappa *et al.*, 1988). In an animal model of alcohol-induced pancreatitis (Kono *et al.*, 2001), rats were kept on diets rich in unsaturated fat and given a high dose of ethanol enterally. Within 4 weeks, the animals showed acinar cell atrophy, fat intiltration in acinar and islet cells, inflammatory cell infiltration and focal necrosis, as well as fibrotic changes, together with a substantial increase in collagen α1(I) mRNA expression. Chronic administration of ethanol resulted in macroscopic and structural abnormalities of B-cells in rats (Koko *et al.*, 1995).

In summary, high doses of ethanol cause pancreatitis in animals, which serves as a model for human pancreatitis.

(iii) *Gastrointestinal tract*

High concentrations of acetaldehyde were found in the colorectal content in piglets after administration of ethanol. Ethanol was oxidized by microbial ADH and acetaldehyde accumulated in high concentrations because ALDH activity was low in the colorectal mucosa of these animals (Jokelainen *et al.*, 1996). The mucosal concentration of acetaldehyde was inversely related to folate levels in the colorectal mucosa of rats that received 3 g/kg bw of ethanol, twice a day for two weeks (Homann *et al.*, 2000b).

In animals that received ethanol in long-term studies, structural alterations indicative of cellular proliferation were observed in the oropharynx and oesophagus, and mucosal atrophy was seen in the oral floor. Pro-inflammatory features such as infiltration of neutrophils and release of reactive oxygen species were noted in the gastric and small intestinal mucosa in rodents shortly after oral or intragastric administration of ethanol (reviewed in Bode & Bode, 2003; Siegmund *et al.*, 2003). Perfusion of jejunal segments of rabbits with 6% (w/v) ethanol caused mucosal injury and enhanced epithelial permeability, which were mediated by the release of radical oxygen species associated with leukocyte infiltration (Dinda *et al.*, 1996). In this study, the ethanol concentration corresponded to the intraluminal concentrations reached in humans

during moderate alcohol consumption (0.8 g/kg bw) (Beck & Dinda, 1981). Gastric mucosal changes associated with chronic ad-libitum ingestion of ethanol comprised epithelial regeneration with enhanced DNA synthesis as a consequence of mucosal injury (Siegmund *et al.*, 2003).

Increased cell proliferation was consistently observed in the large intestine of rodents fed ethanol chronically (Simanowski *et al.*, 1986; 1995). Chronic administration of ethanol via liquid diets led to increased activity of ornithine decarboxylase, a marker enzyme of cell growth and proliferation, in the rectal mucosa of rats (Seitz *et al.*, 1990).

(b) Acetaldehyde

The acute toxicity of acetaldehyde is relatively low: the oral LD_{50} (dose that was lethal to 50% of animals) in rats and mice ranged from 660 to 1930 mg/kg bw and the inhalation LC_{50} (concentration in air that was lethal to 50% of animals) in rats and Syrian hamsters varied from 24 to 37 g/m^3 (IPCS, 1995). Upon repeated dosing by the oral route and inhalation, toxic effects at relatively low concentrations were limited principally to the sites of initial contact. In a 28-day drinking-water study in which acetaldehyde was given to rats at up to 675 mg/kg bw daily for 4 weeks, focal hyperkeratosis of the forestomach was observed at the highest dose (Til *et al.*, 1988). Following inhalation, the respiratory effects seen in rats exposed for 5 weeks and in hamsters exposed for 13 weeks were degenerative changes in the olfactory epithelium (rats, 437 mg/m^3 [243 ppm]; Saldiva *et al.*, 1985) and the trachea (hamsters, 2400 mg/m^3 [1340 ppm]; Kruysse *et al.*, 1975). At higher concentrations, degenerative changes in the respiratory epithelium and larynx were observed.

Effects of acetaldehyde in the liver have been reported at high doses. Intraperitoneal injection of male albino rats with 200 mg/kg bw daily for 10 days caused accumulation in the liver of total lipids, triacyl glycerols and total cholesterol. Other effects were increased glycogenolysis, a shift in metabolism from the citric acid cycle towards the pentose phosphate pathway and an increase in levels of serum triacyl glycerol, total cholesterol and free fatty acids (Prasanna & Ramakrishnan, 1984, 1987). This treatment also altered thyroid function, as indicated by lower serum thyroxine and decreased iodine uptake, but these these effects may have been secondary to the observed hepatic changes (Prasanna *et al.*, 1986). In a similar study with female Sprague-Dawley rats, histopathological changes in the pancreas were noted, with decreased trypsinogen levels and amylase activity (Majumdar *et al.*, 1986).

In a 28-month carcinogenicity study, Wistar rats were exposed by inhalation for 6 h per day on 5 days per week to 1350, 2700 or 5400 mg/m^3 [750, 1500 or 3000 ppm] acetaldehyde. Growth retardation and increased mortality were seen at all dose levels. After one year of treatment, degenerative changes in the olfactory nasal epithelium were observed at each dose level, including slight to severe hyperplasia and keratinized stratified metaplasia of the larynx (high dose only) and degenerative changes of the

upper respiratory epithelium. At the high dose, focal flattening and irregular arrangement of the tracheal epithelium was found. When a subgroup of rats was allowed a 26-week recovery period after 52 weeks of exposure, partial regeneration of the olfactory epithelium was observed in the low- and mid-dose groups (Woutersen *et al.*, 1984, 1986; Woutersen & Feron, 1987).

Tissues that are characterized by rapid cell turnover have an increased susceptibility towards chemical carcinogens; various studies have therefore been performed to evaluate the effect of chronic ethanol consumption on mucosal cell turnover. In rats fed ethanol chronically, the size of the basal-cell nuclei of the oral mucosa from the floor of the mouth, the edge of the tongue and the base of the tongue was significantly enlarged. Chronic ingestion of ethanol also significantly stimulated the production of crypt cells in the rectum. This was associated with an expansion of the proliferative compartment of the crypt, which correlates with an increased risk for rectal cancer. Proliferation rates of crypt cells in the rectum could be correlated with mucosal acetaldehyde concentrations, which would underline a toxic effect of acetaldehyde on the rectal mucosa that induces compensatory hyper-regeneration. These data show that chronic ethanol consumption leads to mucosal hyper-regeneration in the gastrointestinal mucosa associated with an increased risk for cancer. This may therefore represent at least one mechanism by which ethanol exerts its co-carcinogenic effect (Simanowski *et al.*, 1995, 2001).

4.6 Reproductive and perinatal toxicity

4.6.1 *Humans*

(a) Effects on reproduction

The effects of alcoholic beverages on reproduction in both men and women have been reviewed previously (IARC, 1988) and more recently (Emanuele & Emanuele, 1998; Dees *et al.*, 2001; Emanuele *et al.*, 2002).

Alcohol can interfere with the function of each of the components of the male reproductive system, and thereby cause impotence, infertility and reduced male secondary sexual characteristics. In the testes, ethanol can adversely affect the Leydig cells, which produce and secrete testosterone. Heavy alcoholic beverage consumption results in reduced testosterone levels in the blood. Ethanol also impairs the function of the testicular Sertoli cells that play an important role in sperm maturation. In the pituitary gland, ethanol can decrease the production, release and/or activity of two hormones with critical reproductive functions: luteinizing hormone and follicle-stimulating hormone. Finally, ethanol can interfere with hormone production in the hypothalamus (Emanuele & Emanuele, 1998).

It is widely accepted that ethanol also has profound effects on the female reproductive system. Alcohol abuse and alcoholism are associated with a broad spectrum of reproductive system disorders (Mello *et al.*, 1989). Amenorrhoea, anovulation, luteal

phase dysfunction and ovarian pathology may occur in alcohol-dependent women and alcoholic beverage abusers. Luteal phase dysfunction, anovulation and persistent hyperprolactinaemia have also been observed in social drinkers who were studied under clinical research ward conditions. The reproductive consequences of alcohol abuse and alcoholism range from infertility and increased risk for spontaneous abortion to impaired fetal growth and development. It has been suggested that the effects of ethanol on pituitary gonadotropins and on gonadal, steroid and adrenal hormones in women are responsible for these effects (Emanuele *et al.*, 2002). Beyond puberty, ethanol has been found to disrupt normal menstrual cycling in women and to affect hormonal levels in postmenopausal women.

(b) Teratogenic effects

(i) *Transplacental (gestational) exposures*

Ethanol is a well documented human developmental teratogen that can cause a spectrum of physical and mental dysfunctions following prenatal exposure. Multiple terms are used to describe the continuum of effects that result from prenatal exposure to ethanol, the most commonly known of which is fetal alcohol syndrome (FAS).

FAS is a collection of the most severe abnormalities caused by maternal alcohol abuse, and includes pre- and/or postnatal growth retardation, characteristic craniofacial dysmorphology, mental retardation, cardiac septal defects and minor joint abnormalities. Less common features of FAS include abnormalities of multiple organs and systems that encompass vision, hearing and vestibular apparatus, urinary, hepatic, immune and skin defects (Chaudhuri, 2000a,b). Many symptoms of FAS persist well into adulthood (see e.g. Streissguth *et al.*, 1991a).

Abel and Sokol (1987) reported a worldwide incidence of FAS of 1.9 per 1000 live births, and estimated that approximately 6% of the offspring of alcoholic women have FAS. For offspring born after a sibling who had FAS, the risk is much higher (up to 70%; Abel, 1988). The prevalence of FAS is probably considerably underestimated, because of the difficulty in making the diagnosis and the reluctance of clinicians to stigmatize children and mothers (Little & Wendt, 1991; Ceccanti *et al.*, 2004).

A large number of qualitative studies on the prenatal effects of ethanol with resepect to physical and mental development (see, e.g., Coles *et al.*, 1987, Coles, 1993; Larkby & Day, 1997), as well as meta-analytical reviews (Polygenis *et al.*, 1998; Testa *et al.*, 2003), have been undertaken.

Major morphological abnormalities associated with FAS result from exposure early in pregnancy, while growth is most seriously affected by late exposure. Central nervous system deficits occur throughout gestation. Thus, offspring who are exposed to ethanol throughout pregnancy will not have the same outcome as offspring who are exposed only during early pregnancy or only at specific times during pregnancy.

Growth deficits

Children with FAS were reported to have lower body weights than age-matched controls (Streissguth *et al.*, 1991b). FAS-related growth retardation is somewhat ameliorated at puberty. The growth deficits are symmetrical and affect height, weight and head circumference to the same degree, and remain significant until the age of 10 years. The relationship between the intensity of prenatal exposure to alcohol and growth deficits is linear. Smith *et al.* (1986) found that the duration of exposure to alcohol, in addition to the amount consumed, affected birth weight.

Morphological abnormalities

These include facial anomalies, i.e. short palpebral fissures, a flattened nasal bridge, an absent or elongated philtrum and a thin upper lip, which are established when the midline of the face is formed during the first trimester of pregnancy (Day *et al.*, 1990).

Central nervous system deficits

Post-mortem examinations conducted in the late 1970s provided the first evidence of structural brain abnormalities in infants and fetuses of mothers who ingested alcoholic beverages during pregnancy. In addition to microcephaly, the observed malformations included cerebral dysgenesis, *hydrocephalus internus* and hypoplasia or complete agenesis of the olfactory bulbs (Clarren, 1981). In-vivo imaging techniques have been used to examine the brains of children with FAS (Ronen & Andrews, 1991; Mattson *et al.*, 2001; O'Hare *et al.*, 2005). These studies demonstrated ethanol-induced central nervous system dysmorphology that ranged from holoprosencephaly to hypoplasia of specific brain regions. Thus, deficiencies in specific brain structures due to prenatal exposure to ethanol may underlie behavioural and cognitive deficits that are characteristic of FAS (Sowell *et al.*, 2002).

Coles *et al.* (1991) compared the cognitive performance of children whose mothers drank an average of 11.8 oz absolute alcohol (i.e. approximately 24 drinks) per week throughout pregnancy with that in children whose mothers stopped drinking in the second trimester or did not drink at all during pregnancy. At an average age of 5 years and 10 months, children who had been exposed throughout gestation performed more poorly than children in the other two groups, and showed deficits in short-term memory and encoding (i.e. sequential processing) and overall mental processing.

A recent examination of the effects of prenatal exposure to ethanol on the mental development of the infant, as assessed by the mental development index, was conducted in a meta-analysis by Testa *et al.* (2003). This study examined the effects of three levels of average daily exposure during pregnancy: <1 drink per day, 1–1.99 drinks per day and ≥2 drinks per day. Analyses were conducted separately for effects derived from observations of 6–8-, 12–13- and 18–26-month-old children. Fetal exposure to ethanol at all three dosage levels was associated with significantly lower mental development index scores among 12–13-month-olds. For younger and older children, the effect of fetal exposure to ethanol did not attain statistical significance at any dose level.

(ii) *Paternal exposures*

Paternal alcoholic beverage consumption and its effects on the offspring have been reviewed (Abel, 2004).

Tarter *et al.* (1984) compared adolescent sons of alcoholics with sons of non-alcoholics. Using a standardized test of educational achievement, adolescent sons of alcoholics performed significantly worse. Furthermore, it was demonstrated that sons of alcoholics have certain neuropsychological deficits in perceptual-motor ability, memory and language processing. They also had auditory and visual attentional impairments and a lower level of achievement in reading comprehension. In addition, the sons of alcoholics presented a more neurotic personality profile than sons of non-alcoholics.

Savitz *et al.* (1991) analysed data on single live births from 1959 to 1966 among 14 685 Kaiser Foundation Health Plan members to assess the impact of paternal age, cigarette smoking and alcoholic beverage consumption on the occurrence of birth defects in the offspring. Prevalence odds ratios for anomalies identified by age 5 years were analysed, contrasting exposed to unexposed fathers with adjustment for maternal age, race, education, smoking and alcoholic beverage use. Alcoholic beverage use by the father was most positively related to the risk for ventricular septal defects in the offspring but the increase in risk was not significant. These data generally do not indicate strong or widespread associations between paternal attributes and birth defects.

4.6.2 *Experimental systems*

Animal studies dealing with the effects of ethanol on reproduction and fetal development have been reviewed (IARC, 1988; Abel, 2004).

(a) Ethanol

(i) *Effects on reproduction*

In general, animal data have demonstrated decreased litter size, increased prevalence of low-birth-weight fetuses and mixed data on the risk for malformations. Cognitive and behavioural changes that include learning and memory deficits, hyperactivity and poor stress tolerance were found to be the most prominent effects.

(ii) *Teratogenic effects*

Data from the experiments on the transplacental effects of ethanol in animal models, including rodents and non-human primates, largely support the findings in humans. These results have been reviewed extensively (IARC, 1988; Becker *et al.*, 1996b; Goodlett *et al.*, 2005).

(b) Acetaldehyde

Several studies on the developmental effects of acetaldehyde have been conducted, primarily to investigate its role in ethanol-induced teratogenicity (O'Shea & Kaufman,

1979, 1981; Bariliak & Kozachuk, 1983; Webster *et al.*, 1983; Ali & Persaud, 1988). In these studies, acetaldehyde was given by amniotic or intraperitoneal injection, not by ingestion or inhalation. Dose-related embryotoxic, fetotoxic and teratogenic effects were seen in most of these studies, particularly in rats, but maternal toxicity was often not assessed adequately or reported in any of these investigations. Dose-related embryotoxic effects were observed in in-vitro studies on rat embryos exposed to acetaldehyde (Popov *et al.*, 1981; Campbell & Fantel, 1983). Effects on the placenta have been observed following intraperitoneal injection of acetaldehyde into pregnant rats (Sreenathan *et al.*, 1984).

Rat postimplantation embryos at gestation day 9.5 were cultured for 48 h and observed for morphological changes following treatment with acetaldehyde. There was significant cytotoxicity in embryonic midbrain cells. In this tissue, the levels of p53, bcl-2, 8-hydroxydeoxyguanine and the number of cells damaged by reactive oxygen species were increased by the treatment. Co-treatment with acetaldehyde and catalase decreased the cytotoxicity. In postimplantation culture, acetaldehyde-treated embryos showed retardation of embryonic growth and development in a concentration-dependent manner. These results show that acetaldehyde induces fetal developmental abnormalities by disrupting cellular differentiation and growth. Some antioxidants can partially protect against the embryonic developmental toxicity (Lee *et al.*, 2006).

4.7 Genetic and related effects

4.7.1 *Humans*

(a) Ethanol

The genetic and related effects of ethanol in humans published before 1987 have been reviewed previously (IARC, 1988).

More recently, Rajah and Ahuja (1996) evaluated the genotoxicity of a dual exposure to ethanol and lead in workers in the printing industry, and the possible interaction between the two agents. Individuals were classified into four groups: controls, lead-exposed individuals, alcoholic beverage consumers and lead-exposed alcoholic beverage consumers. Alcoholic beverage consumers had a significant increase in the frequency of sister chromatid exchange compared with the controls. Although an increase in the frequency of chromosomal aberrations and sister chromatid exchange was observed in individuals exposed to lead, this increase was not significant. Lead-exposed alcohol consumers had a significant increase in the frequency of chromosomal aberrations and sister chromatid exchange. Statistical analysis did not reveal an interaction between ethanol and lead in either assay.

Maffei *et al.* (2000, 2002) found that the frequency of chromosomal aberrations and micronucleated lymphocytes was significantly higher in 20 alcoholics than in 20 controls. In the alcoholics, no association was found between duration of alcoholic beverage abuse and frequency of genetic damage. In a cytogenetic study with peripheral

blood lymphocytes of 29 chronic alcoholics, 11 alcoholics in abstinence and 10 controls (Burim *et al.*, 2004), the frequencies of chromosomal aberrations for chronic alcoholics and alcoholics in abstinence were higher than those observed in control individuals. The frequencies of chromosomal aberrations seen in alcoholics in abstinence were similar to those obtained for chronic alcoholics. Interestingly, this study found that chromosomal aberrations were not statistically different when smoking and nonsmoking alcoholics were compared, which indicated a lack of interaction. In contrast, several other studies (Castelli *et al.*, 1999; Karaoğuz *et al.*, 2005) reported that the frequency of ethanol-induced sister chromatid exchange, micronucleus formation and chromosomal aberrations was higher in alcoholic beverage abusers who also smoked than in those who did not.

While the majority of the literature shows no increase in the genetic effects of ethanol following abstinence from alcohol drinking, some studies reported conflicting results (De Torok, 1972; Matsushima, 1987). Gattás and Saldanha (1997) compared the frequency of structural and/or numerical chromosomal aberrations in cultures of lymphocytes obtained from alcoholics who were abstinent for between 1 month and 32 years with those from controls who were selected because they did not consume alcoholic beverages. Cytogenetic analyses showed a significant increase of the frequencies of cells with structural aberrations in the abstinent alcoholics (7.1%) compared with controls (2.4%). The frequency of numerical aberrations showed a significant regression with age in both groups.

There is some indication that ethanol may lead to genetic damage in sperm; however, ethanol is not a unique germ-cell mutagen. Adler and Ashby (1989) re-analysed data from the GeneTox Workgroups of the US Environmental Protection Agency and concluded that while ethanol did show clastogenic and aneuploidy-inducing activity, it was not restricted to germ cells. Robbins *et al.* (1997) investigated the potential contribution of common lifestyle exposures (smoking, coffee and alcoholic beverages) to the aneuploidy load in sperm from 45 healthy male volunteers aged 19–35 years. Alcohol consumption was significantly associated with increased frequencies of aneuploidy XX18, diploidy XY18–18 and the duplication phenotype XX18–18, after controlling for caffeine, smoking and donor age.

An increased level of 8-oxo-deoxyguanine in leukocyte DNA was observed in ALDH2-deficient subjects who consumed alcoholic beverages (Nakajima *et al.*, 1996). However, two other studies (van Zeeland *et al.*, 1999; Lodovici *et al.*, 2000) did not detect any increase in 8-oxo-deoxyguanine levels in relation to alcoholic beverage consumption. A multicentre study in Europe (Bianchini *et al.*, 2001) observed an inverse relationship between alcoholic beverage consumption and levels of 8-oxo-deoxyguanine in DNA from leukocytes.

Frank *et al.* (2004) reported a significant increase in 1,N^6-ethenodeoxyadenosine in seven subjects diagnosed with alcoholic fatty liver and three diagnosed with alcoholic fibrosis. Patients with alcoholic fibrosis had a much higher level of these adducts

than patients with alcoholic fatty liver. [The Working Group noted that no diagnostic criteria were provided for patients identified as 'alcoholic'.]

(b) Acetaldehyde

(i) *DNA adduct formation*

Structures of the DNA adducts that result from acetaldehyde (referred to below) are given in Fig. 4.4.

Fang and Vaca (1997) examined the levels of N^2-ethyldeoxyguanosine (N^2-EtdG) adducts in a group of Swedish alcohol abusers compared with controls. The characteristics of the two groups are given in the Table 4.10. Compared with controls, chronic alcoholics had higher levels of the N^2-EtdG adduct in both lymphocytes and granulocytes. The levels of adduct found in both cell types were in the order of 1 lesion/10^7 nucleotides. [The Working Group noted that the alcoholic subjects were also heavy smokers, whereas the control subjects were not. However, the authors reported that N^2-EtdG levels were undetectable in the DNA sample from the one moderate smoker in the control group, and also stated that no adducts were detectable in samples obtained from five additional heavy smokers (>20 cigarettes/week)]. Similar results were found in mice (see Section 4.7.2(*b*)).

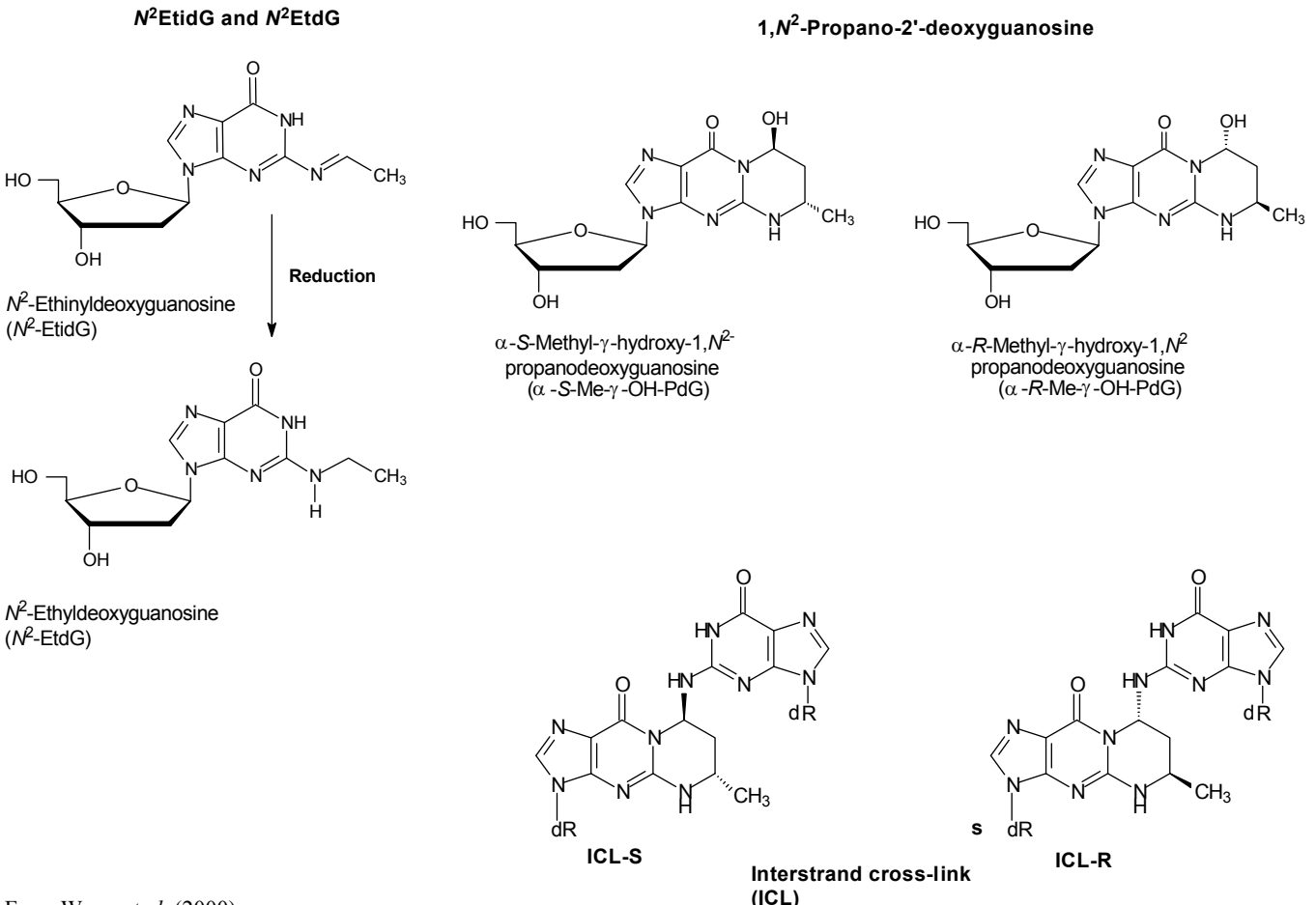
Matsuda *et al.* (2006) analysed the levels of acetaldehyde-derived adducts in DNA samples from the peripheral white blood cells of Japanese alcoholic beverage abusers with two different *ALDH2* genotypes: *2*1/2*1 vs 2*1/2*2* (see Table 4.11). The groups were matched by age, smoking and alcoholic beverage consumption. These authors developed very sensitive and specific liquid chromatography–mass spectrometry assays for three different DNA adducts: N^2-Et-dG, α-methyl-γ-hydroxy-1,N^2-propano-2'-deoxyguanosine (Me-γ-OH-PdG) (both *R* and *S* isomers) and N^2-(2,6-dimethyl-1,3-dioxan-4-yl)-2'-deoxyguanosine (N^2-Dio-dG). The N^2-Dio-dG adduct was not detected in any of the samples studied. However, levels of the other three adducts were significantly higher in *2*1/2*2* carriers than in those with the *2*1/2*1* genotype.

Inclusion of a reducing agent (cyanoborohydride) in the DNA isolation and digestion solutions led to the quantitative conversion of N^2-ethylidene-2'-deoxyguanosine (N^2-EtidG), the major adduct formed by acetaldehyde, to N^2-EtdG. Wang *et al.* (2006) concluded that N^2-EtidG is in fact an endogenous adduct that is present in normal animal and human liver DNA at levels in the range of 0.1 lesion/10^6 normal nucleotides.

Using this methodology, Chen *et al.* (2007) found that the amount of N^2-EtdG in white blood cells showed a small but statistically significant decrease after cessation of smoking, which could be related to a reduction of exposure to acetaldehyde derived from cigarette smoke.

In this study, subjects were eligible to participate only if they normally drank less than six alcoholic beverages per month and abstained from drinking throughout the study. The authors noted that it is difficult to rule out occasional drinking, and therefore

Figure 4.4 DNA adducts that result from acetaldehyde



From Wang *et al.* (2000)

Table 4.10 DNA adducts in alcoholics and controls (characteristics of subjects)

	Controls/moderate drinkers	Alcohol abusers
No. of subjects	12 (8 men, 4 women)	24 (19 men, 5 women)
Median age (range)	32 (25–46) years	46 (31–64) years
Alcohol consumption	None (6 subjects) <50 g/week (6 subjects)	>500 g/week
Smoking	11 nonsmokers 1 moderate smoker (<10 cigarettes/week)	>20 cigarettes/day
DNA-adduct measurements		
Cell type	N^2-*EtdG/10^7 nucleotides*	N^2-*EtdG/10^7 nucleotides*
Granulocytes	Undetectable	3.4±3.8 $p<0.001$
Lymphocytes	0.35 (from 2 subjects; adducts were undetectable in 10 others)	2.1±0.8 $p<0.001$

From Fang & Vaca (1997) EtdG, ethyldeoxyguanosine

no firm conclusions can be drawn from this study about acetaldehyde derived from ethanol metabolism and its role in the formation of this adduct.

Matsuda *et al.* (1999) reported that detectable levels of N^2-EtdG were found in the urine of healthy Japanese individuals who had abstained from ethanol for at least 1 week. These authors proposed that the lesion resulted from endogenously formed acetaldehyde.

Table 4.11 DNA-adduct formation in subjects with different *ALDH2* genotypes

ALDH2 **genotype**	*2*1/2*1*	*2*1/2*2*
No. of subjects	19 men	25 men
Median age (range)	52±11 years	51±11 years
Alcohol consumption	130±54 g/day (910 g/week)	105±59 g/day (735 g/week)
Smoking (cigarettes/day)	22±13	24±15
DNA adducts (fmol/μmol dG)		
N^2-EtdG	17.8±15.9 (adduct detectable in 2/19 samples) 3.9 adducts/10^9 nucleotides[a]	130±52 (p=0.003)* (adduct detectable in 14/25 samples) 28.3 adducts/10^9 nucleotides[a]
α-*S*-Me-γ-OH-PdG	42.9±6.0	92.4±12.9 (p=0.001)*
α-*R*-Me-γ-OH-PdG	61.3±6.4	114±15 (p=0.002)*

From Matsuda *et al*. (2006) ALDH, aldehyde dehydrogenase; dG, deoxyguanosine; EtdG, ethyldeoxyguanosine; Me-γ-OH-PdG, α-methyl-γ-hydroxy-1,N^2-propano-deoxyguanosine * Significantly higher than in *2*1/2*1*; Mann-Whitney U test for N^2-EtdG, t-test for Me-γ-OH-PdG adducts [a] Data converted to adducts/10^9 nucleotides to allow comparison with the study presented in Table 4.10. [The differences probably reflect the greater accuracy from the use of liquid chromatography–mass spectrometry with internal standards by Matsuda *et al*.]

(ii) *Cytogenetic abnormalities in relation to alcoholic beverage consumption*

While studies of chromosomal aberrations in alcoholic beverage abusers do not directly implicate acetaldehyde, these investigations are considered here since numerous other in-vitro studies (see Section 4.7.2(*b*)) have shown that acetaldehyde causes cytogenetic abnormalities in eukaryotic cells *in vitro*. Earlier studies of chromosomal aberrations in the peripheral blood lymphocytes of alcoholics have been reviewed (Obe & Anderson, 1987). The overall results show higher frequencies of chromosomal aberrations (five studies) and sister chromatid exchange (four studies) in alcoholics compared with non-alcoholics. The results of three more recent studies are discussed below, and details are given in Table 4.12. Additional cytogenetic studies in alcoholics are mentioned in Table 4.13.

Gattás and Saldanha (1997) studied chromosomal aberrations in abstinent Brazilian alcoholics *vs* controls (not screened for alcoholic beverage consumption) and observed a significant difference in the percentage of cells with chromosomal aberrations (7.1% for abstinent alcoholics, 2.4% for controls).

Maffei *et al*. (2002) found that alcoholics who consumed >120 g alcohol per day had significantly more chromatid breaks, chromosome breaks, total chromosomal aberrations and cells with micronuclei than either non-drinking controls or abstinent alcoholics. The three groups were matched for age, sex and smoking. These results confirmed those of an earlier study by the same laboratory (Castelli *et al*., 1999). Another study by the same group combined fluorescence *in-situ* hybridization with the analysis of micronucleus formation and showed an increase in the number of cells with micronuclei (Maffei *et al*., 2000).

In a combined analysis of three different studies, Iarmarcovai *et al*. (2007) observed a small but significant increase in micronucleus formation in alcoholic beverage users compared with controls (odds ratio, 1.24; 95% CI, 1.01–1.53).

(iii) *Other data on genetic toxicology in alcoholic beverage abusers*

Pool-Zobel *et al*. (2004) used the comet assay to assess DNA damage and repair in human rectal cells obtained from biopsies. Unexpectedly, they observed that male alcoholic beverage abusers had significantly less genetic damage than male controls. [The authors suggested that this may be the result of an enhancing effect on endogenous defence, e.g. through upregulation of DNA repair in response to damage. Alternatively, a reduced amount of DNA in the comet tails could reflect DNA–protein cross-links resulting from exposure to endogenous acetaldehyde.]

4.7.2 *Experimental systems*

(a) Ethanol

The genotoxic potential of ethanol has been evaluated extensively in lower organisms, plants, mammalian systems and in human cells. Ethanol is generally considered

Table 4.12 Recent studies of chromosomal aberrations/micronuclei in human alcoholics

Reference, study location	Characteristics of subjects	Characteristics of controls	Matching factors	Alcohol consumption	Tissue and genetic biomarker	Results	Comments
Gattás & Saldanha (1997), Brazil	45 men (41.8± 9.2 years old), 10 women (37.9±10 years old) from an Alcoholics Anonymous group	31 men (36.5±9.2 years old), 24 women (31.5±7.5 years old) not screened for alcohol	Age	19.1 years of drinking (range 6–35 years); 46 months of abstinence (range, 1–384 months)	Peripheral blood lymphocytes; chromosomal aberrations	7.1% of cells with aberrations in abstinent alcoholics versus 2.4% in controls $p<0.0001$	Significantly greater numbers of aberrations in >5 years versus <5 years of abstinence, but effect confounded by age difference
Maffei *et al*. (2002), Italy	20 alcoholics, 20 abstinent alcoholics; several clinical tests administered to rule out a general state of malnutrition in alcoholics	20 controls	Age, sex, smoking	Controls: none; alcoholics: alcohol abuse for 19.5±8.8 years (range, 4–40 years) >120 g/day; abstinent alcoholics: >120g/day for at least 5 years before quitting, abstinent for 32.5±15.5 months	Peripheral blood lymphocytes; chromosomal aberrations, binucleated cells with MN	Alcoholics had significantly more chromitid breaks, chromosome breaks, total chromosome aberrations and binucleated cells with MN than either controls or abstinent alcoholics.	Consistent with results from earlier study by same group showing increased chromosomal aberrations and MN in alcoholics, and reversibility in abstinence. Earlier study (Castelli *et al*., 1999) did not match for age or smoking

Table 4.12 (continued)

Reference, study location	Characteristics of subjects	Characteristics of controls	Matching factors	Alcohol consumption	Tissue and genetic biomarker	Results	Comments
Iarmarcovai *et al*. (2007), France, Italy	Pooled analysis from three independent studies; 10 cancer patients; 27 welders; 18 pathologists/ anatomists; 50 alcohol drinkers obtained from within these groups	10 controls; 30 unexposed controls; 18 controls; 54 non-drinking controls	Age, sex		Peripheral blood lymphocytes; micronuclei	For alcohol drinkers versus non-drinkers; frequency ratios (95% CI) from multiple regression analysis; total MN, 1.24 (1.01–1.53); one centromere-+ MN, 1.29 (1.01–1.65); one centromere-+ MN, 1.42 (1.07–1.89)	

CI, confidence interval; MN, micronuclei

Table 4.13 Genetic and related effects of alcohol/ethanol

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli K-12 *uvrB/recA*, differential toxicity	–	–	78200	Hellmér & Bolcsfoldi (1992)
Salmonella typhimurium TA100, TA104, TA1535, TA98, TA97, reverse mutation	–	–	10 mg/plate	Zeiger *et al.* (1992)
Salmonella typhimurium TA100, TA1535, TA1537, TA97, TA98, reverse mutation	–	–	5–10 mg/plate	Phillips & Jenkinson (2001)
Saccaromyces cerevisiae, (repair-deficient) strand breaks	+	NT	39100	Ristow *et al*. (1995)
Aspergillus nidulans, chromosome malsegregation	+	NT	35500	Crebelli *et al*. (1989)
Vicia faba, sister chromatid exchange	+	NT	16000	Zhang *et al*. (1991)
Hordeum species, sister chromatid exchange	+	NT	16000	Zhang *et al*. (1991)
Plant (other), sister chromatid exchange	+	NT	16000	Zhang *et al*. (1991)
Drosophila melanogaster, somatic mutation (and recombination)	–	NT	120000	Graf *et al*. (1994)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	(+)	4200	Wangenheim & Bolcsfoldi (1988)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	35900	Phillips & Jenkinson (2001)
Sister chromatid exchange, mouse embryos *in vitro*	+	NT	300	Lau *et al.* (1991)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	–	8000	Phillips & Jenkinson (2001)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	NT	32000	Lin *et al.* (1989)
Chromosomal aberrations, mouse embryos *in vitro*	+	NT	800	Lau *et al.* (1991)
DNA strand breaks, human lymphocytes *in vitro*	+	NT	1380	Blasiak *et al*. (2000)
DNA strand breaks, human colonic mucosa *in vitro*	+	NT	460	Blasiak *et al*. (2000)
DNA strand breaks, human gastric mucosa *in vitro*	+	NT	46000	Blasiak *et al*. (2000)
Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	40000	Zhang *et al*. (1991)

Table 4.13 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	–	–	8000	Phillips & Jenkinson (2001)
Chromosomal aberrations, human lymphoid cell lines *in vitro*	–	NT	32000	Hsu *et al.* (1991)
Chromosomal aberrations, human lymphoblast cell lines *in vitro*	–	NT	8000	Brown *et al.* (1991)
DNA adducts, BD_6 rat tissues *in vivo*	–		4300	Izzotti *et al.* (1998)
DNA strand breaks, rat brain cells *in vivo*	+		4000	Singh *et al.* (1995)
DNA strand breaks, Wistar rat liver cells *in vivo*	+		5000	Navasumrit *et al.* (2000)
Sister chromatid exchange, mouse cells *in vivo*	+		1600	Zhang *et al.* (1991)
Sister chromatid exchange, mouse bone marrow *in vivo*	+		600	Piña Calva & Madrigal-Bujaidar (1993)
Micronucleus formation, B6C3F1 mouse spermatids *in vivo*	–		28500	Pylkkänen & Salonen (1987)
Micronucleus formation, BD_6 rat bone-marrow cells and pulmonary alveolar macrophages *in vivo*	–		50 g/L in drinking-water	Balansky *et al.* (1993)
Micronucleus formation, CD-1 mouse polychromatic erythrocytes *in vivo*	–		3500	Choy *et al.* (1995)
Micronucleus formation, CD-1 mouse polychromatic erythrocytes *in vivo*	–		2500	Choy *et al.* (1996)
Micronucleus formation, mouse *in vivo*	–		2000	Phillips & Jenkinson (2001)
Chromosomal aberrations, Wistar rat bone marrow *in vivo*	–		200 g/L in drinking-water	Tavares *et al.* (2001)
Aneuploidy, Chinese hamster spermatogonia *in vivo*	–		6250	Daniel & Roane (1987)
Aneuploidy, (C57BL x CBA) F_1 Mouse oocytes *in vivo*	+		4800	O'Neill & Kaufman (1987)

Table 4.13 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal test, mice	(+)		1260 × 3	Rao *et al.* (1994)
Dominant lethal test, mice	+		25000	Berryman *et al.* (1992)
Studies on alcoholics				
Gene mutation, human lymphocytes, *HPRT* locus *in vivo*	–			Cole & Green (1995)
Sister chromatid exchange, human lymphocytes *in vivo*	+			Butler *et al.* (1981)
Sister chromatid exchange, human lymphocytes *in vivo*	(+)			Seshadri *et al.* (1982)
Sister chromatid exchange, human lymphocytes *in vivo*	+			Kucheria *et al.* (1986)
Sister chromatid exchange, human lymphocytes *in vivo*	+			Rajah & Ahuja (1996)
Sister chromatid exchange, human lymphocytes *in vivo*	+[c]			Karaoğuz *et al.* (2005)
Micronucleus formation, human buccal mucosa cells *in vivo*	–			Stich & Rosin (1983)
Micronucleus formation, human buccal epithelium *in vivo*	+			Ramirez & Saldanha (2002)
Micronucleus formation, human lymphocytes *in vivo*	+ [c]			Castelli *et al.* (1999)
Micronucleus formation, human lymphocytes *in vivo*	+			Maffei *et al.* (2000)
Micronucleus formation, human lymphocytes *in vivo*	+			Maffei *et al.* (2002)
Micronucleus formation, human lymphocytes *in vivo*	(+)			Ishikawa *et al.* (2006)
Chromosomal aberrations, human lymphocytes *in vivo*	+			De Torok (1972)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Lilly (1975)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Mitelman & Wadstein (1978)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Obe *et al.* (1980)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Badr & Hussain (1982)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Kucheria *et al.* (1986)
Chromosomal aberrations, human lymphocytes *in vivo*	–			Rajah & Ahuja (1996)

Table 4.13 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vivo*	+			Gattás & Saldanha (1997)
Chromosomal aberrations, human lymphocytes *in vivo*	+[c]			Castelli *et al*. (1999)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Hüttner *et al*. (1999)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Maffei *et al*. (2002)
Chromosomal aberrations, human lymphocytes *in vivo*	+			Burim *et al*. (2004)
Aneuploidy, human sperm *in vivo*	+			Robbins *et al*. (1997)

[a] +, positive; (+), weak positive; –, negative; NT, not tested [b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day [c] In these studies, people who consumed alcohol were also heavy smokers.

to be non-mutagenic. The genotoxicity data for ethanol have been reviewed (IARC, 1988; Phillips & Jenkinson, 2001). The activity profile of alcohol in short-term genotoxicity tests published since the previous monograph is shown in Table 4.13 (with references) and summarized below.

The available published data from genotoxicity tests of ethanol in bacteria and *Drosophila* largely show that it is not a mutagen, even in the presence of exogenous metabolic activation systems. This was also confirmed in studies that used ethanol as a vehicle control in assays that involved these organisms, which suggests that it is not mutagenic or clastogenic *in vitro*. Ethanol caused anomalous chromosome segregation in *Aspergillus*, DNA strand-breaks in yeast, and chromosomal aberrations and sister chromatid exchange in plants.

In human and mammalian cells *in vitro*, ethanol generally did not induce genetic damage; however, it induced sister chromatid exchange and chromosomal aberrations in preimplantation mouse embryos cultured *in vitro*. In human lymphocytes and lymphoblastoid cells *in vitro*, most of the evidence showed no effect of ethanol in these assays. In animals *in vivo*, ethanol induced a variety of genetic effects, including DNA strand breaks, induction of sister chromatid exchange and dominant lethal mutations. Several studies showed no effect of ethanol in the micronucleus assay. Strain-dependent differences in the activity of ethanol in the dominant lethal assay in rodents have been reported.

In studies in rats, exposure to ethanol leads to alterations in the structural and functional integrity of hepatic mitochondria, to increased mitochondrial DNA oxidation and to a decrease in the amount of mitochondrial DNA (Cahill *et al*., 1997, 2005). Several studies showed that administration of ethanol to rats and mice leads to changes in activity and amount of DNA-repair proteins in the liver (Navasumrit *et al*., 2001a; Bradford *et al*., 2005).

Several types of DNA damage have been associated with administration of ethanol to rats, which leads to the accumulation of DNA single-strand breaks in liver parenchymal cells, an effect that closely matched the timing of CYP2E1 induction and was inhibited by dietary antioxidants (Navasumrit *et al*., 2000). An increase in the lipid peroxidation-derived DNA adduct, ethenodeoxycytidine, was seen in rats given a single dose of ethanol (5 g/kg bw) or a 1-week treatment with ethanol (5% w/v) in a liquid diet (Navasumrit *et al*., 2001b). Fang and Vaca (1995) found that exposure of mice to 10% (v/v) ethanol in the drinking-water for five weeks resulted in levels of 1.5±0.8 (n=7) N^2-EtdG/10^8 nucleotides in liver DNA. Adducts were undetectable in control mice. Bradford *et al*. (2005) found that rats and mice exposed to ethanol by intragastric feeding (14–28 g/kg bw per day for 28 days) showed increased levels of oxidative DNA damage (abasic sites and 8-hydroxydeoxyguanine) in the liver. In the same study and under the same conditions of ethanol administration, these effects were observed in transgenic mice that expressed human CYP2E1, but not in *CYP2E1*-knockout mice or in the presence of a CYP2E1 inhibitor.

(b) Acetaldehyde (see Table 4.14)

(i) *DNA adduct formation*

N^2-Ethyl-2′-deoxyguanosine (N^2-EtdG)

The most abundant adduct that results from the reaction of acetaldehyde with DNA is N^2-EtidG (see Fig. 4.4). This adduct is too unstable for purification, but can be converted to a stable adduct, N^2-EtdG, by treatment with a reducing agent (sodium cyanoborohydride). *In vitro*, the reduction step can also be carried out by a mixture of GSH and ascorbic acid, which may reflect *in vivo* conditions (Wang *et al.*, 2006; see also Fang & Vaca, 1995).

Other acetaldehyde-derived DNA adducts

In addition to the major adduct, N^2-EtidG (and N^2-EtdG after reduction with borohydride), three additional acetaldehyde-derived DNA adducts have been identified. These are: N^2-Dio-dG, an interstrand cross-link, and two diasteresmers (*R* and *S*) of Me-α-OH-PdG (see Fig. 4.4). (Wang *et al.*, 2000).

The formation of the Me-α-OH-PdG adducts can be facilitated by including either basic amino acids, histones (which are rich in basic amino acids), or polyamines in the reaction mixture. In the presence of physiologically relevant polyamine concentrations, detectable amounts of these adducts were formed at concentrations as low as 100 μM acetaldehyde (Theruvathu *et al.*, 2005). Such concentrations are within the range of those formed in the saliva of human volunteers who drank alcoholic beverage in a laboratory setting (Homann *et al.*, 1997). Finally, acetaldehyde can react with malondialdehyde, and the resulting conjugate can form DNA adducts *in vitro* (Pluskota-Karwatka *et al.*, 2006).

(ii) *Mutagenic activity of acetaldehyde-derived DNA adducts*

The mutagenic potential of specific DNA adducts can be tested with single-stranded DNA vectors that contain a single adduct located within a reporter gene. These constructs can then be transfected into cells, allowed to replicate and the resulting replication products analysed for mutations by various methods, depending on the specific nature of the reporter gene. Using such an approach, the N^2-EtdG adduct was only minimally mutagenic to the *supF* gene in the reporter plasmid pLSX (mean mutant fraction, 0.9±0.2% for the adduct-containing construct vs 0.4±0.2% for the lesion-free control) when replicated in *E. coli* (P=0.09). When deoxyuridines were placed on the complementary strand at 5′ and 3′ positions flanking the adduct, the mutant fractions increased to 1.4±0.5% for the lesion vs 0.6±4% for the control (P=0.04) (Upton *et al.*, 2006). [It should be pointed out that this study was carried out with N^2-EtdG, whereas, *in vivo*, most probably the N^2-EtidG adduct is formed predominantly.]

Two separate studies have shown that Me-α-OH-PdG adducts result in mutant fractions of 5–11% when inserted in a shuttle vector and replicated in either monkey kidney cells (Fernandes *et al.*, 2005) or SV40-transformed human fibroblasts (Stein *et al.*, 2006). In both cases, the predominant mutagenic event observed was a G→T

Table 4.14 Genetic and related effects of acetaldehyde

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
*Escherichia coli pol*A, differential toxicity (spot test)	(+)	NT	10 µL/plate	Rosenkranz (1977)
Escherichia coli K-12 *uvrB/recA*, differential toxicity	–	NT	16300	Hellmér & Bolcsfoldi (1992)
Salmonella typhimurium TA100, TA1535, TA1537, TA98 reverse mutation	–	–	3333 µg/plate	Mortelmans *et al*. (1986)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	0.5% in air	JETOC (1997)
Salmonella typhimurium TA102, TA104, reverse mutation	–	NT	1 mg/plate	Marnett *et al*. (1985)
Salmonella typhimurium TA1535, reverse mutation	–	NT	10 µL/plate	Rosenkranz (1977)
Salmonella typhimurium TA1538, reverse mutation	–	NT	10 µL/plate	Rosenkranz (1977)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	0.5% in air	JETOC (1997)
Aspergillus nidulans, aneuploidy (chromosome malsegregation)	+	NT	200	Crebelli *et al*. (1989)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		22500 ppm inj × 1	Woodruff *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		25000 ppm feed, 3 d	Woodruff *et al*. (1985)
DNA–protein cross-links, Fischer 344 rat nasal mucosa cells *in vitro*	+	NT	4400	Lam *et al*. (1986)
DNA–protein cross-links, plasmid DNA and histones, *in vitro*	+	NT	440	Kuykendall & Bogdanffy (1992)
Comet assay, cultured rat neurons *in vitro*	+		11	Lamarche *et al.* (2004)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	176	Wangenheim & Bolcsfoldi (1988)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	3.9	Obe & Ristow (1977)

Table 4.14 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.9	Obe & Beek (1979)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	7.8	de Raat *et al*. (1983)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.3	Brambilla *et al*. (1986)
Micronucleus formation, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	22	Bird *et al*. (1982)
Chromosomal aberrations, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	4.4	Bird *et al*. (1982)
Chromosomal aberrations, Chinese hamster embryonic diploid fibroblasts *in vitro*	+	NT	31	Dulout & Furnus (1988)
Cell transformation, C3H 10T½ mouse cells	–[c]	NT	100	Abernethy *et al*. (1982)
Cell transformation, rat kidney cells	–[c]	NT	132	Eker & Sanner (1986)
DNA strand breaks, human lymphocytes *in vitro*, alkaline elution	–	NT	440	Lambert *et al*. (1985)
DNA cross-links, human lymphocytes *in vitro*, alkaline elution	+	NT	440	Lambert *et al*. (1985)
DNA strand breaks and DNA–protein cross-links, human bronchial epithelial cells *in vitro*	–	NT	44	Saladino *et al*. (1985)
DNA strand breaks, human lymphocytes *in vitro*	+	NT	68.8	Singh & Khan (1995)
Comet assay, cultured human lymphocytes *in vitro*	+		132	Blasiak *et al.* (2000)
Comet assay, cultured colonic and gastric mucosa *in vitro*	+		4400	Blasiak *et al.* (2000)
Gene mutation, human lymphocytes, *HPRT* locus *in vitro*	+	NT	11	He & Lambert (1990)

Table 4.14 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	7.9	Obe *et al*. (1978); Ristow & Obe (1978)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4	Jansson (1982)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	15.9	Böhlke *et al*. (1983)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	He & Lambert (1985)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	Knadle (1985); Helander & Lindahl-Kiessling (1991)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	11	Norppa *et al*. (1985); Sipi *et al*. (1992)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	15.9	Obe *et al*. (1986)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	20	Badr & Hussain (1977)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	15.9	Obe *et al*. (1979)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	31.7	Böhlke *et al*. (1983)
Chromosomal aberrations, human Fanconi's anaemia lymphocytes *in vitro*	+	NT	7.9	Obe *et al*. (1979)
Micronucleus formation, human lymphocytes *in vitro*	+[d]		26.4	Migliore *et al*. (1996)
Micronucleus formation, human HepG2 and Hep3B cells *in vitro*	+	NT	39.6	Majer *et al.* (2004)
DNA–protein cross-links, Fischer 344 rat nasal mucosa *in vivo*	+	–	1000 ppm inh 6 h/d × 5 d	Lam *et al*. (1986)
Sister chromatid exchange, male C3A mouse bone-marrow cells *in vivo*	+		0.4 µg/mouse ip × 1	Obe *et al*. (1979)
Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		0.5 mg/kg ip × 1	Korte *et al*. (1981)

Table 4.14 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, male C3A mouse bone-marrow cells *in vivo*	+		40 mg/kg ip × 1	Torres-Bezauri *et al.* (2002)
Micronucleus formation, C57BL/6J × C3H/He mouse spermatocytes *in vivo*	–		375 mg/kg ip × 1	Lähdetie (1988)
Chromosomal aberrations, rat embryos *in vivo*	+		158 µg iam × 1	Bariliak & Kozachuk (1983)
N^2-EtdG adduct formation, human buccal cells, *in vitro*	+		440	Vaca et al. (1995)
N^2-EtdG adduct formation, calf thymus DNA *in vitro*	+		72100	Fang & Vaca (1995)
N^2-EtdG adduct formation, deoxynucleosides *in vitro*	+		158580	Vaca *et al.* (1995)
PdG adduct formation, pig liver DNA *in vitro* (in presence of polyamines)	+		4.4	Theruvathu *et al.* (2005)
PdG adduct formation, calf thymus DNA *in vitro* (in presence of histones)	+		26430	Sako *et al.* (2003)
Binding (covalent) to calf thymus DNA *in vitro*	+	NT	44050	Ristow & Obe (1978)
Binding (covalent) to deoxynucleosides *in vitro*	+	NT	158580	Vaca *et al.* (1995)
Sperm morphology, C57BL/6J × C3H/He mouse early spermatids *in vivo*	–		250 ip × 5	Lähdetie (1988)

EtdG, ethyldeoxyguanosine; PdG, 1,N^2-propanodeoxyguanosine

[a] +, positive; (+), weak positive; –, negative; NT, not tested [b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; d, day; iam, intra-amniotic; inh, inhalation; inj, injection; ip, intraperitoneal [c] Positive results when acetaldehyde treatment was followed by exposure of the cells to 12-*O*-tetradecanoylphorbol 13-acetate: 10 µg/mL (Abernethy *et al.*, 1982), 10^{-5}M (Eker & Sanner, 1986) [d] A dose-related increase in centromere-positive micronuclei was observed with fluorescence in-situ hybridization but it was not significantly different from the negative control.

transversion, but G→A and G→C mutations were also found. In comparison, the ethenodeoxyadenosine adduct resulted in mutant fractions as a high as 70% in COS7 monkey kidney cells (Pandya & Moriya, 1996), but the mutant fraction was only 7–14% in human cells (Levine *et al.*, 2000). Methodological differences, differences in the host cells used or in the local sequence in the shuttle vectors may be responsible for the different results.

An important feature of the deoxyguanosine adducts, which is not shared by N^2-EtidG or N^2-EtdG, is that they can undergo ring-opening when located in double-stranded DNA (Mao *et al.*, 1999). The ring-opened forms of the Me-α-OH-PdG adducts can react with proteins to generate DNA–protein cross-links (Kurtz & Lloyd, 2003). With a deoxyguanosine residue in the opposite strand of the helix, a DNA–intrastrand cross-link can be formed (Wang *et al.*, 2000). Intrastrand cross-links generated in this manner are also mutagenic (mutant fraction, 3–6%) in mammalian cells, and generate primarily G→T transversions, as well as deletion and insertion mutations (Liu *et al.*, 2006). Matsuda *et al.* (1998) exposed plasmid DNA that contains a *supF* mutation reporter gene to concentrations of acetaldehyde up to 1M, and allowed the plasmid to replicate in human XP-A cells, which are deficient in nucleotide excision repair. In contrast to the results for Me-α-OH-PdG adducts, these authors observed GG→TT mutations. The DNA lesions responsible for these mutations are most probably not propano-deoxyguanosine adducts, but the intrastrand cross-links.

4.8 Mechanistic considerations

4.8.1 *Ethanol*

The mechanisms of the induction of cancer by consumption of alcoholic beverages and more specifically ethanol are not entirely clear, and are certainly complex. In this section some of the diverse effects that could contribute to ethanol-induced carcinogenesis are discussed.

(a) Tumour initiation

(i) *Molecular genetic epidemiology of ethanol-metabolizing systems (see Section 4.3)*

The role of the metabolism of ethanol in carcinogenesis associated with alcoholic beverage consumption is suggested by several positive associations between different forms of cancer and certain polymorphisms in genes that are involved in the activation of ethanol. The degree to which these associations are explained by acetaldehyde production, redox changes, formation of radicals, effects on intermediary metabolism and/or effects on other pro-carcinogens can not be established from current findings. However, the results of these studies strongly indicate a prominent role for acetaldehyde, the primary metabolite of ethanol.

(ii) *Oxidative stress*

Ethanol promotes the production of reactive oxygen species both directly, through the formation of the α-hydroxyethyl radical, and indirectly, via induction of oxidative stress. Oxidative stress results from ethanol metabolism, tissue inflammation and increased iron storage. Ethanol-induced CYP2E1 produces various reactive oxygen species, which lead to the formation of lipid peroxides such as 4-hydroxy-nonenal. Furthermore, ethanol impairs the antioxidant defence system, which results in enhanced mitochondrial damage and apoptosis. Alcoholic beverage consumption leads to the activation of resident macrophages in the liver (Kupffer cells) and to the recruitment of other immune cells that are capable of producing reactive oxygen and nitrogen species. Increased iron overload of certain tissues has also been reported following alcoholic beverage intake, which may lead to the exacerbation of oxidative stress through iron-mediated production of radicals by the Fenton reaction. DNA damage is the outcome of increased oxidative stress that is associated with ethanol-induced carcinogenesis in many organs. Direct damage results from the metabolism of ethanol to acetaldehyde, which can damage DNA and inhibit DNA-repair systems. Indirect DNA damage is the result of increased production of oxidants and DNA-reactive lipid peroxides that can form carcinogenic DNA adducts (reviewed by Seitz & Stickel, 2006).

(iii) *Toxicokinetics*

Ethanol modifies the toxicokinetics and toxicodynamics of other chemicals (see Section 4.4). It has major effects on the metabolism and clearance of a variety of carcinogens and toxicants, including nitrosamines, urethane, vinyl chloride, benzene and many other solvents. These chemicals are ubiquitous in food, tobacco, air and occupational settings, and at least one nitrosamine, NDMA, is generated endogenously. The effects of ethanol on the metabolism of these substances are therefore of general interest as a potential element in the mechanism of alcohol-induced carcinogenesis. Although ethanol may in theory potentiate the tissue-specific effects of carcinogens by inducing CYP-dependent activation, most findings indicate that a predominant mechanism is competitive inhibition of clearance of the carcinogens, especially in the liver, which results in increased dose delivery to peripheral target organs, with a consequent increase in DNA damage and tumour initiation. Such effects are often quite large: fivefold increases are common, and up to 20-fold enhancements have been observed. Competitive inhibition by ethanol of CYP2E1 is the best understood, but ethanol also inhibits human CYP1A1, -2B6 and -2C19 (reviewed by Lieber *et al.*, 1987; Swann *et al.*, 1987; Anderson *et al.*, 1995).

(b) Tumour promotion

(i) *Ethanol-mediated tumour promotion*

Ethanol has been purported to have tumour-promoting abilities. Several studies in experimental animals have shown that administration of ethanol reduces the latency

of tumour development after treatment with genotoxic carcinogens. Several possible pathways have been suggested to account for this apparent promotional activity. First, the cytotoxicity of ethanol may induce regenerative growth, which increases cell-proliferation rates in affected tissues. Activation of the innate immune response in organs affected by ethanol, such as the liver, has been well documented and this may result in the production of mitogenic cytokines. In addition, treatment with ethanol leads to excess production of oxygen free radicals and lipid peroxidation. An increase in lipid peroxidation was observed in the liver as well as other tissues that were targets for site-specific carcinogens. This process was enhanced by ethanol. An increase in arachidonate and an over-production of polyunsaturated fatty acids involved in eicosanoid synthesis have also been reported as a consequence of treatment with ethanol and may play a key role in excessive cell proliferation and selective outgrowth of initiated cells (reviewed by Mufti, 1998).

(ii) *Induction of mitogen-activated protein kinases (MAPK)*

Ethanol induces expression of inhibitory G-proteins which in turn activate the mitogen-activated protein kinase (MAPK) -signalling cascade that is essential in the initiation of cell proliferation and differentiation, apoptosis, stress and inflammatory responses. Acute exposure to ethanol gives rise to modest activation of p42/44 MAPK in hepatocytes, astrocytes and vascular smooth muscle cells. Acute and chronic exposure to ethanol also results in potentiation or prolonged activation of MAPK in an agonist-selective manner, especially in innate immune cells that promote inflammation and tissue damage. Ethanol-induced activation of MAPK-signalling is also involved in collagen expression in hepatic stellate cells, and thus promotes liver fibrosis and cirrhosis. Some of the effects of ethanol on MAPK-signalling are thought to be mediated by acetaldehyde, rather than by ethanol itself (reviewed by Aroor & Shukla, 2004).

(iii) *Vitamin A (retinol)*

Retinoic acid plays an important role in controlling cell growth, differentiation and apoptosis. Alcoholic beverage consumption is associated with a decrease in hepatic levels of vitamin A, a precursor of retinoic acid. Thus, it has been suggested that ethanol-induced changes in retinoic acid levels in tissues will lead to impairment of retinoic acid-dependent signalling pathways, interference of 'cross-talk' with MAPK cascades and disturbances in cell-cycle regulation that may lead to carcinogenesis. Several possible mechanisms for the interaction between ethanol and retinoic acid have been proposed. Ethanol may act as a competitive inhibitor of the oxidation of vitamin A to retinoic acid that involves ADHs and ALDHs; ethanol-induced CYP enzymes, particularly CYP2E1, may enhance catabolism of vitamin A and retinoic acid; and ethanol may alter retinoid homeostasis by increasing vitamin A mobilization from the liver to extrahepatic tissues (reviewed by Leo & Lieber, 1999; Wang, 2005).

(iv) *Insulin-like growth factors (IGFs)*

The insulin-like growth factors (IGFs) are mitogens that play a pivotal role in the regulation of cell proliferation, differentiation and apoptosis. Their effects are mediated through the IGF-I receptor, which is also involved in cell transformation induced by tumour virus proteins and oncogene products. It has been suggested that ethanol-induced carcinogenesis, e.g., in the breast, is associated with effects on IGFs, but the relationship between alcoholic beverage consumption and IGF levels is unclear. Different patterns of alcoholic beverage consumption may have opposite effects on IGF levels. Long-term and heavy drinking can cause severe damage to the liver, and loss of liver function may result in a decline in the production of IGFs. Alcoholics are reported to have relatively low levels of IGF-I, but, in animal studies, ethanol enhanced the action and expression of IGF-I (reviewed by Yu & Berkel, 1999; Yu & Rohan, 2000) .

(v) *Folate and DNA methylation (reviewed in Section 4.3)*

Folate deficiency is associated with different forms of cancer, of which colon cancer is the most commonly described. Ethanol *per se* and an underlying unhealthy lifestyle associated with high alcoholic beverage consumption are known to cause folate deficiency, which increases the risk for cancer. The degree to which the relation between alcohol drinking, folate deficiency and cancer may be explained by the metabolism of ethanol is not known.

(vi) *Ethanol and sex hormones*

Estrogens and androgens are well known activators of cellular proliferation, which is associated with an increased risk for carcinogenesis. Alcoholic beverage use in women causes an increase in the levels of estrogen and/or androgen, which may promote the development of breast cancer (reviewed by Gavaler, 1995; Singletary & Gapstur, 2001; Dumitrescu & Shields, 2005).

(vii) *Cirrhosis*

Ethanol causes hepatocellular injury that can lead to enhanced fibrogenesis and finally cirrhosis. Liver cirrhosis is strongly associated with an increased risk for hepatocellular carcinoma. Ethanol-related hepatocellular carcinoma without pre-existing cirrhosis is rare, which indicates that the pathogenic events that lead to cirrhosis precede those that cause cancer, or that the structural alterations in the liver during cirrhosis, together with other factors, favour the transformation of hepatocytes (reviewed by Stickel *et al.*, 2002; Seitz & Stickel, 2006)

(c) Tumour progression

(i) *Immunodeficiency and immunosuppression*

Alcoholic beverage drinking increases immunodeficiency and immunosuppression, conditions that may facilitate carcinogenesis by silencing immune-related defence mechanisms in various organs. It is widely recognized that chronic alcoholics are more

susceptible to infections and to certain neoplasms. The following factors related to alcoholism affect the immune system: malnutrition, vitamin deficiencies, established cirrhosis and ethanol itself. The suppression by ethanol of natural killer cells, which are implicated in the control of tumour development and growth, has been shown in cultured cells, animal studies and in human alcoholics. Although there is general agreement on the impact of alcohol consumption on the immune system, the mechanisms by which ethanol compromises anti-tumour immune surveillance are not yet known completely (reviewed by Watson *et al.*, 1992; Cook, 1998; Stickel *et al.*, 2002).

4.8.2 *The role of acetaldehyde in alcohol-induced carcinogenesis*

Over the past 10 years, epidemiological evidence of enhanced cancer risks among heterozygous carriers of the inactive allele of the ALDH2 enzyme has become much stronger, in particular for oesophageal cancer: all nine case–control studies conducted in Japan among independent populations who consumed alcoholic beverages show significantly increased odds ratios (range, 3.7–13.5) for carriers of the inactive *ALDH2* allele. These data suggest that acetaldehyde is the key metabolite in the development of oesophageal cancer associated with alcoholic beverage consumption in these populations. The mechanistic considerations that support this suggestion can be summarized as follows: (*a*) there is a causal relationship between alcoholic beverage consumption and cancer in the oral cavity, pharynx, larynx, oesophagus and liver; (*b*) it is generally accepted that ethanol in alcoholic beverages is the principal ingredient that renders these beverages carcinogenic; (*c*) in the body, ethanol is converted by ADH to acetaldehyde, which is oxidized by ALDH to acetate; (*d*) the formation of acetaldehyde starts in the mouth (mediated by oral bacteria) and continues along the digestive tract; production of acetaldehyde is also found in the liver and in the gut. This largely parallels the target organ sites known to date to be susceptible to ethanol-induced cancer. Given its volatile nature, it is conceivable that ingested acetaldehyde reaches the respiratory tract; (*e*) acetaldehyde is a cytotoxic, genotoxic, mutagenic and clastogenic compound. It is carcinogenic in experimental animals; (*f*) after alcoholic beverage consumption, carriers of an inactive allele of the ALDH2 enzyme show accumulating levels of acetaldehyde in the peripheral blood, which is a direct consequence of their enzyme deficiency, and show increased levels of N^2-EtdG and Me-α-OH-PdG adducts in lymphocyte DNA. The latter adducts have been shown to be formed from acetaldehyde; during DNA replication, these adducts cause mutations; (*g*) consumers of alcoholic beverages have a higher frequency of chromosomal aberrations, sister chromatid exchange and micronucleus formation in the peripheral lymphocytes than control nondrinkers. These effects may be attributable to acetaldehyde, which is a clastogen; (*h*) several of the observations made in ALDH2-deficient individuals have been confirmed in *ALDH2*-knockout mice.

In view of these considerations, the Working Group concluded that acetaldehyde, the primary metabolite of ethanol, is the carcinogen that leads to the formation of oesophageal cancer in carriers of the inactive *ALDH2* allele who consume alcoholic beverages.

References

Abel E (2004). Paternal contribution to fetal alcohol syndrome. *Addict Biol*, 9: 127–133, discussion 135–136. doi:10.1080/13556210410001716980 PMID:15223537

Abel EL (1988). Fetal alcohol syndrome in families. *Neurotoxicol Teratol*, 10: 1–2. doi:10.1016/0892-0362(88)90060-8 PMID:3352564

Abel EL & Sokol RJ (1987). Incidence of fetal alcohol syndrome and economic impact of FAS-related anomalies. *Drug Alcohol Depend*, 19: 51–70. doi:10.1016/0376-8716(87)90087-1 PMID:3545731

Abernethy DJ, Frazelle JH, Boreiko CJ (1982). Effects of ethanol, acetaldehyde and acetic acid in the C3H/10T1/2 cl8 cell transformation system. *Environ Mol Mutagen*, 4: 331

Adachi M & Ishii H (2002). Role of mitochondria in alcoholic liver injury. *Free Radic Biol Med*, 32: 487–491. doi:10.1016/S0891-5849(02)00740-2 PMID:11958949

Adler ID & Ashby J (1989). The present lack of evidence for unique rodent germ-cell mutagens. *Mutat Res*, 212: 55–66. PMID:2725542

Adler RA (1992). Clinical review 33: Clinically important effects of alcohol on endocrine function. *J Clin Endocrinol Metab*, 74: 957–960. doi:10.1210/jc.74.5.957 PMID:1569170

Agarwal DP (2001). Genetic polymorphisms of alcohol metabolizing enzymes. *Pathol Biol (Paris)*, 49: 703–709. PMID:11762132

Agarwal DP (2002). Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol Alcohol*, 37: 409–415. PMID:12217928

Albano E (2006). Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc*, 65: 278–290. doi:10.1079/PNS2006496 PMID:16923312

Ali F & Persaud TV (1988). Mechanisms of fetal alcohol effects: role of acetaldehyde. *Exp Pathol*, 33: 17–21. PMID:3384064

Allali-Hassani A, Martinez SE, Peralba JM *et al*. (1997). Alcohol dehydrogenase of human and rat blood vessels. Role in ethanol metabolism. *FEBS Lett*, 405: 26–30. doi:10.1016/S0014-5793(97)00151-8 PMID:9094418

Altomare E, Grattagliano I, Vendemiale G *et al*. (1996). Acute ethanol administration induces oxidative changes in rat pancreatic tissue. *Gut*, 38: 742–746. doi:10.1136/gut.38.5.742 PMID:8707122

Ammon E, Schäfer C, Hofmann U, Klotz U (1996). Disposition and first-pass metabolism of ethanol in humans: is it gastric or hepatic and does it depend on gender? *Clin Pharmacol Ther*, 59: 503–513. doi:10.1016/S0009-9236(96)90178-2 PMID:8646821

Anandatheerthavarada HK, Shankar SK, Bhamre S *et al.* (1993). Induction of brain cytochrome P-450IIE1 by chronic ethanol treatment. *Brain Res*, 601: 279–285. doi:10.1016/0006-8993(93)91721-4 PMID:8431773

Anderson LM (1988). Increased numbers of N-nitrosodimethylamine-initiated lung tumors in mice by chronic co-administration of ethanol. *Carcinogenesis*, 9: 1717–1719. doi:10.1093/carcin/9.9.1717 PMID:3409476

Anderson LM (1992) Modulation of nitrosamine metabolism by ethanol: implications for cancer risk. In: Watson, R.R., ed., *Alcohol and Cancer*, Boca Raton, FL, CRC Press, pp. 17–54.

Anderson LM, Carter JP, Driver CL *et al.* (1993). Enhancement of tumorigenesis by N-nitrosodiethylamine, N-nitrosopyrrolidine and N6-(methylnitroso)-adenosine by ethanol. *Cancer Lett*, 68: 61–66. doi:10.1016/0304-3835(93)90220-4 PMID:8422650

Anderson LM, Carter JP, Logsdon DL *et al.* (1992a). Characterization of ethanol's enhancement of tumorigenesis by N-nitrosodimethylamine in mice. *Carcinogenesis*, 13: 2107–2111. doi:10.1093/carcin/13.11.2107 PMID:1423883

Anderson LM, Chhabra SK, Nerurkar PV *et al.* (1995). Alcohol-related cancer risk: a toxicokinetic hypothesis. *Alcohol*, 12: 97–104. doi:10.1016/0741-8329(94)00089-1 PMID:7772272

Anderson LM, Koseniauskas R, Burak ES *et al.* (1992b). Reduced blood clearance and increased urinary excretion of N-nitrosodimethylamine in patas monkeys exposed to ethanol or isopropyl alcohol. *Cancer Res*, 52: 1463–1468. PMID:1540953

Anderson LM, Koseniauskas R, Burak ES *et al.* (1994). Suppression of in vivo clearance of N-nitrosodimethylamine in mice by cotreatment with ethanol. *Drug Metab Dispos*, 22: 43–49. PMID:8149888

Anderson LM, Souliotis VL, Chhabra SK *et al.* (1996). *N*-Nitrosodimethylamine-derived O^6-methylguanine in DNA of monkey gastrointestinal and urogenital organs and enhancement by ethanol. *Int J Cancer*, 66: 130–134. doi:10.1002/(SICI)1097-0215(19960328)66:1<130::AID-IJC22>3.0.CO;2-G PMID:8608956

Aoki Y & Itoh H (1989). Effects of alcohol consumption on mitochondrial aldehyde dehydrogenase isoenzymes in rat liver. *Enzyme*, 41: 151–158. PMID:2721481

Apte MV & Wilson JS (2003). Alcohol-induced pancreatic injury. *Best Pract Res Clin Gastroenterol*, 17: 593–612. doi:10.1016/S1521-6918(03)00050-7 PMID:12828957

Aragno M, Tamagno E, Danni O *et al.* (1996). In vivo potentiation of 1,2-dibromoethane hepatotoxicity by ethanol through inactivation of glutathione-S-transferase. *Chem Biol Interact*, 99: 277–288. doi:10.1016/0009-2797(95)03678-4 PMID:8620575

Ardies CM, Smith TJ, Kim S, Yang CS (1996). Induction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) activation in rat lung microsomes by chronic ethanol consumption and repeated running exercise. *Cancer Lett*, 103: 209–218. doi:10.1016/0304-3835(96)04216-4 PMID:8635159

Aroor AR & Shukla SD (2004). MAP kinase signaling in diverse effects of ethanol. *Life Sci*, 74: 2339–2364. doi:10.1016/j.lfs.2003.11.001 PMID:15027449

Arteel GE, Raleigh JA, Bradford BU, Thurman RG (1996). Acute alcohol produces hypoxia directly in rat liver tissue in vivo: role of Kupffer cells. *Am J Physiol*, 271: G494–G500. PMID:8843775

Asakage T, Yokoyama A, Haneda T *et al.* (2007). Genetic polymorphisms of alcohol and aldehyde dehydrogenases, and drinking, smoking and diet in Japanese men with oral and pharyngeal squamous cell carcinoma. *Carcinogenesis*, 28: 865–874. doi:10.1093/carcin/bgl206 PMID:17071628

Asami S, Hirano T, Yamaguchi R *et al.* (2000). Increase in 8-hydroxyguanine and its repair activity in the esophagi of rats given long-term ethanol and nutrition-deficient diet. *Jpn J Cancer Res*, 91: 973–978. PMID:11050466

Asmussen E, Hald J, Larsen V (1948). The pharmacological action of acetaldehyde on the human organism. *Acta Pharmacol.*, 4: 311–320.

Aze Y, Toyoda K, Furukawa F *et al.* (1993). Enhancing effect of ethanol on esophageal tumor development in rats by initiation of diethylnitrosamine. *Carcinogenesis*, 14: 37–40. doi:10.1093/carcin/14.1.37 PMID:8425269

Baarson KA, Snyder CA, Green JD *et al.* (1982). The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow, and spleen cells are increased by ingested ethanol. *Toxicol Appl Pharmacol*, 64: 393–404. doi:10.1016/0041-008X(82)90235-6 PMID:7135393

Badger TM, Hoog J-O, Svensson S *et al.* (2000). Cyclic expression of class I alcohol dehydrogenase in male rats treated with ethanol. *Biochem Biophys Res Commun*, 274: 684–688. doi:10.1006/bbrc.2000.3186 PMID:10924336

Badger TM, Huang J, Ronis M, Lumpkin CK (1993). Induction of cytochrome P450 2E1 during chronic ethanol exposure occurs via transcription of the CYP 2E1 gene when blood alcohol concentrations are high. *Biochem Biophys Res Commun*, 190: 780–785. doi:10.1006/bbrc.1993.1117 PMID:8439329

Badger TM, Ronis MJJ, Frank SJ *et al.* (2003). Effects of chronic ethanol on hepatic and renal CYP2C11 in the male rat: interactions with the Janus-kinase 2-signal transducer and activators of transcription proteins 5b pathway. *Endocrinology*, 144: 3969–3976. doi:10.1210/en.2002-0163 PMID:12933671

Badr FM & Hussain F (1977). Action of ethanol and its metabolite acetaldehyde in human lymphocytes: in vivo and in vitro study. *Genetics*, 86: S2–S3.

Badr FM & Hussain FH (1982). Chromosomal aberrations in chronic male alcoholics. *Alcohol Clin Exp Res*, 6: 122–129. doi:10.1111/j.1530-0277.1982.tb05390.x PMID:7041680

Balansky RM, Blagoeva PM, Mircheva ZI, de Flora S (1993). Coclastogenicity of ethanol with cigarette smoke in rat erythroblasts and anticlastogenicity in alveolar macrophages. *Cancer Lett*, 72: 183–189. doi:10.1016/0304-3835(93)90127-U PMID:8402590

Baraona E, Abittan CS, Lieber CS (2000). Contribution of gastric oxidation to ethanol first-pass metabolism in baboons. *Alcohol Clin Exp Res*, 24: 946–951. PMID:10923995

Bardag-Gorce F, French BA, Li J *et al.* (2002). The importance of cycling of blood alcohol levels in the pathogenesis of experimental alcoholic liver disease in rats. *Gastroenterology*, 123: 325–335. doi:10.1053/gast.2002.34177 PMID:12105860

Bariliak IR & Kozachuk SIu (1983). Embryotoxic and mutagenic activity of ethanol and acetaldehyde in intra-amniotic exposure *Tsitol Genet*, 17: 57–60. PMID:6649057

Barja G & Herrero A (2000). Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J*, 14: 312–318. PMID:10657987

Beck IT & Dinda PK (1981). Acute exposure of small intestine to ethanol: effects on morphology and function. *Dig Dis Sci*, 26: 817–838. doi:10.1007/BF01309614 PMID:7285748

Becker HC, Diaz-Granados JL, Randall CL (1996b). Teratogenic actions of ethanol in the mouse: a minireview. *Pharmacol Biochem Behav*, 55: 501–513. doi:10.1016/S0091-3057(96)00255-9 PMID:8981580

Becker U, Deis A, Sørensen TIA *et al.* (1996a). Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. *Hepatology*, 23: 1025–1029. doi:10.1002/hep.510230513 PMID:8621128

Berryman SH, Anderson RA Jr, Weis J, Bartke A (1992). Evaluation of the co-mutagenicity of ethanol and delta 9-tetrahydrocannabinol with Trenimon. *Mutat Res*, 278: 47–60. doi:10.1016/0165-1218(92)90285-8 PMID:1370119

Bianchini F, Jaeckel A, Vineis P *et al.* (2001). Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine in humans. *Carcinogenesis*, 22: 885–890. doi:10.1093/carcin/22.6.885 PMID:11375894

Bird RP, Draper HH, Basrur PK (1982). Effect of malonaldehyde and acetaldehyde on cultured mammalian cells. Production of micronuclei and chromosomal aberrations. *Mutat Res*, 101: 237–246. doi:10.1016/0165-1218(82)90155-0 PMID:7087985

Błasiak J (2001). Ethanol and acetaldehyde impair the repair of bleomycin-damaged DNA in human lymphocytes. *Cytobios*, 106: Suppl 2141–149. PMID:11545442

Blasiak J, Trzeciak A, Malecka-Panas E *et al.* (2000). In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells. *Toxicol In Vitro*, 14: 287–295. doi:10.1016/S0887-2333(00)00022-9 PMID:10906435

Board P, Smith S, Green J *et al.* (1993). Evidence against a relationship between fatty acid ethyl ester synthase and the Pi class glutathione S-transferase in humans. *J Biol Chem*, 268: 15655–15658. PMID:8340390

Boccia S, De Lauretis A, Gianfagna F *et al.* (2007). CYP2E1PstI/RsaI polymorphism and interaction with tobacco, alcohol and GSTs in gastric cancer susceptibility: a meta-analysis of the literature. *Carcinogenesis*, 28: 101–106. doi:10.1093/carcin/bgl124 PMID:16837478

Bode C & Bode JC (2003). Effect of alcohol consumption on the gut. *Best Pract Res Clin Gastroenterol*, 17: 575–592. doi:10.1016/S1521-6918(03)00034-9 PMID:12828956

Boffetta P & Hashibe M (2006). Alcohol and cancer. *Lancet Oncol*, 7: 149–156. doi:10.1016/S1470-2045(06)70577-0 PMID:16455479

Bogdanffy MS, Randall HW, Morgan KT (1986). Histochemical localization of aldehyde dehydrogenase in the respiratory tract of the Fischer-344 rat. *Toxicol Appl Pharmacol*, 82: 560–567. doi:10.1016/0041-008X(86)90291-7 PMID:3952738

Böhlke JU, Singh S, Goedde HW (1983). Cytogenetic effects of acetaldehyde in lymphocytes of Germans and Japanese: SCE, clastogenic activity, and cell cycle delay. *Hum Genet*, 63: 285–289. doi:10.1007/BF00284666 PMID:6852826

Boleda MD, Julià P, Moreno A, Parés X (1989). Role of extrahepatic alcohol dehydrogenase in rat ethanol metabolism. *Arch Biochem Biophys*, 274: 74–81. doi:10.1016/0003-9861(89)90416-5 PMID:2774584

Boonyaphiphat P, Thongsuksai P, Sriplung H, Puttawibul P (2002). Lifestyle habits and genetic susceptibility and the risk of esophageal cancer in the Thai population. *Cancer Lett*, 186: 193–199. doi:10.1016/S0304-3835(02)00354-3 PMID:12213289

Bora PS, Bora NS, Wu XL, Lange LG (1991). Molecular cloning, sequencing, and expression of human myocardial fatty acid ethyl ester synthase-III cDNA. *J Biol Chem*, 266: 16774–16777. PMID:1885604

Bora PS, Guruge BL, Miller DD *et al.* (1996). Purification and characterization of human heart fatty acid ethyl ester synthase/carboxylesterase. *J Mol Cell Cardiol*, 28: 2027–2032. doi:10.1006/jmcc.1996.0195 PMID:8899561

Bosron WF, Crabb DW, Housinger TA, Li T-K (1984). Effect of fasting on the activity and turnover of rat liver alcohol dehydrogenase. *Alcohol Clin Exp Res*, 8: 196–200. doi:10.1111/j.1530-0277.1984.tb05837.x PMID:6375431

Bosron WF & Li T-K (1986). Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology*, 6: 502–510. doi:10.1002/hep.1840060330 PMID:3519419

Bouchardy C, Hirvonen A, Coutelle C *et al.* (2000). Role of alcohol dehydrogenase 3 and cytochrome P-4502E1 genotypes in susceptibility to cancers of the upper aerodigestive tract. *Int J Cancer*, 87: 734–740. doi:10.1002/1097-0215(20000901)87:5<734::AID-IJC17>3.0.CO;2-E PMID:10925369

Bradford BU, Kono H, Isayama F *et al.* (2005). Cytochrome P450 CYP2E1, but not nicotinamide adenine dinucleotide phosphate oxidase, is required for ethanol-induced oxidative DNA damage in rodent liver. *Hepatology*, 41: 336–344. doi:10.1002/hep.20532 PMID:15660387

Bradford BU & Rusyn I (2005). Swift increase in alcohol metabolism (SIAM): understanding the phenomenon of hypermetabolism in liver. *Alcohol*, 35: 13–17. doi:10.1016/j.alcohol.2004.12.001 PMID:15922133

Bradford BU, Seed CB, Handler JA *et al.* (1993). Evidence that catalase is a major pathway of ethanol oxidation in vivo: dose-response studies in deer mice using meth-

anol as a selective substrate. *Arch Biochem Biophys*, 303: 172–176. doi:10.1006/abbi.1993.1269 PMID:8489262

Brambilla G, Sciabà L, Faggin P *et al.* (1986). Cytotoxicity, DNA fragmentation and sister-chromatid exchange in Chinese hamster ovary cells exposed to the lipid peroxidation product 4-hydroxynonenal and homologous aldehydes. *Mutat Res*, 171: 169–176. doi:10.1016/0165-1218(86)90051-0 PMID:3755798

Brayton RG, Stokes PE, Schwartz MS, Louria DB (1970). Effect of alcohol and various diseases on leukocyte mobilization, phagocytosis and intracellular bacterial killing. *N Engl J Med*, 282: 123–128. doi:10.1056/NEJM197001152820303 PMID:4982606

Brennan DF, Betzelos S, Reed R, Falk JL (1995). Ethanol elimination rates in an ED population. *Am J Emerg Med*, 13: 276–280. doi:10.1016/0735-6757(95)90199-X PMID:7755817

Brennan P, Lewis S, Hashibe M *et al.* (2004). Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer: a HuGE review. *Am J Epidemiol*, 159: 1–16. doi:10.1093/aje/kwh003 PMID:14693654

Britton A & McKee M (2000). The relation between alcohol and cardiovascular disease in Eastern Europe: explaining the paradox. *J Epidemiol Community Health*, 54: 328–332. doi:10.1136/jech.54.5.328 PMID:10814651

Brooks PJ & Theruvathu JA (2005). DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. *Alcohol*, 35: 187–193. doi:10.1016/j.alcohol.2005.03.009 PMID:16054980

Brown AS, Fiatarone JR, Wood P *et al.* (1995). The effect of gastritis on human gastric alcohol dehydrogenase activity and ethanol metabolism. *Aliment Pharmacol Ther*, 9: 57–61. doi:10.1111/j.1365-2036.1995.tb00352.x PMID:7766745

Brown J, King A, Francis CK (1991). Cardiovascular effects of alcohol, cocaine, and acquired immune deficiency. *Cardiovasc Clin*, 21: 341–376. PMID:2044115

Burim RV, Canalle R, Takahashi CS *et al.* (2004). Clastogenic effect of ethanol in chronic and abstinent alcoholics. *Mutat Res*, 560: 187–198. PMID:15157656

Burnell JC, Bosron WF (1989) Genetic polymorphism of human liver alcohol dehydrogenase and kinetic properties of the isoenzymes. In *Human metabolism of alcohol, Vol. 2: Regulation, enzymology and metabolism of ethanol* (K. E. Crow and R. D. Batt, Eds.), CRC Press, Boca Raton F.L. pp. 65–75.

Busby WF Jr, Ackermann JM, Crespi CL (1999). Effect of methanol, ethanol, dimethyl sulfoxide, and acetonitrile on in vitro activities of cDNA-expressed human cytochromes P-450. *Drug Metab Dispos*, 27: 246–249. PMID:9929510

Butler MG, Sanger WG, Veonett GE (1981). Increased frequency of sister-chromatid exchanges in alcoholics. *Mutat Res*, 85: 71–76. PMID:7266565

Caballería J (1992). First-pass metabolism of ethanol: its role as a determinant of blood alcohol levels after drinking. *Hepatogastroenterology*, 39: Suppl 162–66. PMID:1349554

Caballeria J, Baraona E, Lieber CS (1987). The contribution of the stomach to ethanol oxidation in the rat. *Life Sci*, 41: 1021–1027. doi:10.1016/0024-3205(87)90691-6 PMID:3613852

Caballeria J, Baraona E, Rodamilans M, Lieber CS (1989a). Effects of cimetidine on gastric alcohol dehydrogenase activity and blood ethanol levels. *Gastroenterology*, 96: 388–392. PMID:2910758

Caballeria J, Frezza M, Hernández-Muñoz R *et al.* (1989b). Gastric origin of the first-pass metabolism of ethanol in humans: effect of gastrectomy. *Gastroenterology*, 97: 1205–1209. PMID:2792658

Cahill A, Cunningham CC, Adachi M *et al.* (2002). Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. *Alcohol Clin Exp Res*, 26: 907–915. PMID:12068261

Cahill A, Hershman S, Davies A, Sykora P (2005). Ethanol feeding enhances age-related deterioration of the rat hepatic mitochondrion. *Am J Physiol Gastrointest Liver Physiol*, 289: G1115–G1123. doi:10.1152/ajpgi.00193.2005 PMID:16020655

Cahill A, Wang X, Hoek JB (1997). Increased oxidative damage to mitochondrial DNA following chronic ethanol consumption. *Biochem Biophys Res Commun*, 235: 286–290. doi:10.1006/bbrc.1997.6774 PMID:9199183

Cai L, You N-CY, Lu H *et al.* (2006). Dietary selenium intake, aldehyde dehydrogenase-2 and X-ray repair cross-complementing 1 genetic polymorphisms, and the risk of esophageal squamous cell carcinoma. *Cancer*, 106: 2345–2354. doi:10.1002/cncr.21881 PMID:16639733

Caldecott KW (2003). DNA single-strand break repair and spinocerebellar ataxia. *Cell*, 112: 7–10. doi:10.1016/S0092-8674(02)01247-3 PMID:12526788

Campbell MA & Fantel AG (1983). Teratogenicity of acetaldehyde in vitro: relevance to the fetal alcohol syndrome. *Life Sci*, 32: 2641–2647. doi:10.1016/0024-3205(83)90355-7 PMID:6855459

Cao C, Leng Y, Liu X *et al.* (2003). Catalase is regulated by ubiquitination and proteosomal degradation. Role of the c-Abl and Arg tyrosine kinases. *Biochemistry*, 42: 10348–10353. doi:10.1021/bi035023f PMID:12950161

Carlson GP (1994). The effect of inducers and inhibitors of urethane metabolism on its in vitro and in vivo metabolism in rats. *Cancer Lett*, 87: 145–150. doi:10.1016/0304-3835(94)90215-1 PMID:7812933

Caro AA & Cederbaum AI (2004). Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol*, 44: 27–42. doi:10.1146/annurev.pharmtox.44.101802.121704 PMID:14744237

Carr LG, Kirchner J, Magnes L *et al.* (1995). Rat mitochondrial aldehyde dehydrogenase polymorphism and major histocompatibility complex RT1.A phenotypes are not associated with alcohol drinking in P and NP rats. *Alcohol Clin Exp Res*, 19: 447–451. doi:10.1111/j.1530-0277.1995.tb01529.x PMID:7625580

Carrière V, Berthou F, Baird S *et al.* (1996). Human cytochrome P450 2E1 (CYP2E1): from genotype to phenotype. *Pharmacogenetics*, 6: 203–211. doi:10.1097/00008571-199606000-00002 PMID:8807659

Carroccio A, Wu D, Cederbaum AI (1994). Ethanol increases content and activity of human cytochrome P4502E1 in a transduced HepG2 cell line. *Biochem Biophys Res Commun*, 203: 727–733. doi:10.1006/bbrc.1994.2242 PMID:8074729

Castelli E, Hrelia P, Maffei F *et al.* (1999). Indicators of genetic damage in alcoholics: reversibility after alcohol abstinence. *Hepatogastroenterology*, 46: 1664–1668. PMID:10430317

Castellsagué X, Quintana MJ, Martínez MC *et al.* (2004). The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. *Int J Cancer*, 108: 741–749. doi:10.1002/ijc.11627 PMID:14696101

Castonguay A, Rivenson A, Trushin N *et al.* (1984). Effects of chronic ethanol consumption on the metabolism and carcinogenicity of N′-nitrosonornicotine in F344 rats. *Cancer Res*, 44: 2285–2290. PMID:6722769

Castro GD, de Castro CR, Maciel ME *et al.* (2006). Ethanol-induced oxidative stress and acetaldehyde formation in rat mammary tissue: potential factors involved in alcohol drinking promotion of breast cancer. *Toxicology*, 219: 208–219. doi:10.1016/j.tox.2005.11.019 PMID:16377051

Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA (2001a). Cytosolic xanthine oxidoreductase mediated bioactivation of ethanol to acetaldehyde and free radicals in rat breast tissue. Its potential role in alcohol-promoted mammary cancer. *Toxicology*, 160: 11–18. doi:10.1016/S0300-483X(00)00433-9 PMID:11246119

Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA (2001b). Rat ventral prostate xanthine oxidase bioactivation of ethanol to acetaldehyde and 1-hydroxyethyl free radicals: analysis of its potential role in heavy alcohol drinking tumor-promoting effects. *Teratog Carcinog Mutagen*, 21: 109–119. doi:10.1002/1520-6866(2001)21:2<109::AID-TCM1>3.0.CO;2-4 PMID:11223889

Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA (2002). Rat ventral prostate microsomal biotransformation of ethanol to acetaldehyde and 1-hydroxyethyl radicals: its potential contribution to prostate tumor promotion. *Teratog Carcinog Mutagen*, 22: 335–341. doi:10.1002/tcm.10028 PMID:12210496

Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA (2003). Rat breast microsomal biotransformation of ethanol to acetaldehyde but not to free radicals: its potential role in the association between alcohol drinking and breast tumor promotion. *Teratog Carcinog Mutagen*, Suppl 161–70. doi:10.1002/tcm.10060 PMID:12616597

Ceccanti M, Romeo M, Fiorentino D (2004). Alcohol and women: clinical aspects *Ann Ist Super Sanita*, 40: 5–10. PMID:15269446

Cederbaum AI (2003). Iron and CYP2E1-dependent oxidative stress and toxicity. *Alcohol*, 30: 115–120. doi:10.1016/S0741-8329(03)00104-6 PMID:12957295

Cederbaum AI (2006). CYP2E1–biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt Sinai J Med*, 73: 657–672. PMID:16878272

Chao Y-C, Liou S-R, Chung Y-Y *et al.* (1994). Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. *Hepatology*, 19: 360–366. doi:10.1002/hep.1840190214 PMID:7904979

Chao Y-C, Wang L-S, Hsieh T-Y *et al.* (2000). Chinese alcoholic patients with esophageal cancer are genetically different from alcoholics with acute pancreatitis and liver cirrhosis. *Am J Gastroenterol*, 95: 2958–2964. doi:10.1111/j.1572-0241.2000.02328.x PMID:11051375

Chaudhuri JD (2000a). Alcohol and the developing fetus–A review. *Med Sci Monit*, 6: 1031–1041. PMID:11208451

Chaudhuri JD (2000b). An analysis of the teratagenic effects that could possibly be due to alcohol consumption by pregnant mothers. *Indian J Med Sci*, 54: 425–431. PMID:11262858

Chaves ACS, Fernandez M, Lerayer ALS *et al.* (2002). Metabolic engineering of acetaldehyde production by Streptococcus thermophilus. *Appl Environ Microbiol*, 68: 5656–5662. doi:10.1128/AEM.68.11.5656-5662.2002 PMID:12406762

Chen CC, 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*, 65: 795–807. doi:10.1086/302540 PMID:10441588

Chen CS & Yoshida A (1991). Enzymatic properties of the protein encoded by newly cloned human alcohol dehydrogenase ADH6 gene. *Biochem Biophys Res Commun*, 181: 743–747. doi:10.1016/0006-291X(91)91253-9 PMID:1755855

Chen H-J, Tian H, Edenberg HJ (2005a). Natural haplotypes in the regulatory sequences affect human alcohol dehydrogenase 1C (ADH1C) gene expression. *Hum Mutat*, 25: 150–155. doi:10.1002/humu.20127 PMID:15643610

Chen J, Gammon MD, Chan W *et al.* (2005b). One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. *Cancer Res*, 65: 1606–1614. doi:10.1158/0008-5472.CAN-04-2630 PMID:15735051

Chen J, Giovannucci E, Kelsey K *et al.* (1996). A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*, 56: 4862–4864. PMID:8895734

Chen J, Hunter DJ, Stampfer MJ *et al.* (2003). Polymorphism in the thymidylate synthase promoter enhancer region modifies the risk and survival of colorectal cancer. *Cancer Epidemiol Biomarkers Prev*, 12: 958–962. PMID:14578129

Chen J, Kyte C, Chan W *et al.* (2004). Polymorphism in the thymidylate synthase promoter enhancer region and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev*, 13: 2247–2250. PMID:15598787

Chen L, Wang M, Villalta PW *et al.* (2007). Quantitation of an acetaldehyde adduct in human leukocyte DNA and the effect of smoking cessation. *Chem Res Toxicol*, 20: 108–113. doi:10.1021/tx060232x PMID:17226933

Chen Y-J, Chen C, Wu D-C *et al.* (2006). Interactive effects of lifetime alcohol consumption and alcohol and aldehyde dehydrogenase polymorphisms on esophageal cancer risks. *Int J Cancer*, 119: 2827–2831. doi:10.1002/ijc.22199 PMID:17036331

Chern M-K & Pietruszko R (1995). Human aldehyde dehydrogenase E3 isozyme is a betaine aldehyde dehydrogenase. *Biochem Biophys Res Commun*, 213: 561–568. doi:10.1006/bbrc.1995.2168 PMID:7646513

Chhabra SK, Anderson LM, Perella C *et al.* (2000). Coexposure to ethanol with N-nitrosodimethylamine or 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone during lactation of rats: marked increase in O^6-methylguanine–DNA adducts in maternal mammary gland and in suckling lung and kidney. *Toxicol Appl Pharmacol*, 169: 191–200. doi:10.1006/taap.2000.9068 PMID:11097872

Chhabra SK, Souliotis VL, Harbaugh JW *et al.* (1995). O6-Methylguanine DNA adduct formation and modulation by ethanol in placenta and fetal tissues after exposure of pregnant patas monkeys to N-nitrosodimethylamine. *Cancer Res*, 55: 6017–6020. PMID:8521384

Chhabra SK, Souliotis VL, Kyrtopoulos SA, Anderson LM (1996). Nitrosamines, alcohol, and gastrointestinal tract cancer: recent epidemiology and experimentation. *In Vivo*, 10: 265–284. PMID:8797028

Choi J-Y, Abel J, Neuhaus T *et al.* (2003). Role of alcohol and genetic polymorphisms of CYP2E1 and ALDH2 in breast cancer development. *Pharmacogenetics*, 13: 67–72. doi:10.1097/00008571-200302000-00002 PMID:12563175

Chou W-Y, Stewart MJ, Carr LG *et al.* (1999). An A/G polymorphism in the promoter of mitochondrial aldehyde dehydrogenase (ALDH2): effects of the sequence variant on transcription factor binding and promoter strength. *Alcohol Clin Exp Res*, 23: 963–968. doi:10.1111/j.1530-0277.1999.tb04213.x PMID:10397279

Choy WN, Black W, Mandakas G *et al.* (1995). A pharmacokinetic study of ethanol inhibition of micronuclei induction by urethane in mouse bone marrow erythrocytes. *Mutat Res*, 341: 255–263. doi:10.1016/0165-1218(95)90097-7 PMID:7531285

Choy WN, Mandakas G, Paradisin W (1996). Co-administration of ethanol transiently inhibits urethane genotoxicity as detected by a kinetic study of micronuclei induction in mice. *Mutat Res*, 367: 237–244. doi:10.1016/S0165-1218(96)90083-X PMID:8628331

Clarkson SG & Wood RD (2005). Polymorphisms in the human XPD (ERCC2) gene, DNA repair capacity and cancer susceptibility: an appraisal. *DNA Repair (Amst)*, 4: 1068–1074. doi:10.1016/j.dnarep.2005.07.001 PMID:16054878

Clarren SK (1981). Recognition of fetal alcohol syndrome. *JAMA*, 245: 2436–2439. doi:10.1001/jama.245.23.2436 PMID:7230482

Cole J & Green MH (1995). Absence of evidence for mutagenicity of alcoholic beverages: an analysis of HPRT mutant frequencies in 153 normal humans. *Mutagenesis*, 10: 449–452. doi:10.1093/mutage/10.5.449 PMID:8544760

Coles CD (1993). Impact of prenatal alcohol exposure on the newborn and the child. *Clin Obstet Gynecol*, 36: 255–266. doi:10.1097/00003081-199306000-00007 PMID:8513623

Coles CD, Brown RT, Smith IE *et al.* (1991). Effects of prenatal alcohol exposure at school age. I. Physical and cognitive development. *Neurotoxicol Teratol*, 13: 357–367. doi:10.1016/0892-0362(91)90084-A PMID:1921915

Coles CD, Smith IE, Falek A (1987). Prenatal alcohol exposure and infant behavior: immediate effects and implications for later development. *Adv Alcohol Subst Abuse*, 6: 87–104. PMID:3425480

Cook RT (1998). Alcohol abuse, alcoholism, and damage to the immune system–A review. *Alcohol Clin Exp Res*, 22: 1927–1942. PMID:9884135

Corrao G, Rubbiati L, Bagnardi V *et al.* (2000). Alcohol and coronary heart disease: a meta-analysis. *Addiction*, 95: 1505–1523. doi:10.1046/j.1360-0443.2000.951015056.x PMID:11070527

Coutelle C, Höhn B, Benesova M *et al.* (2004). Risk factors in alcohol associated breast cancer: alcohol dehydrogenase polymorphism and estrogens. *Int J Oncol*, 25: 1127–1132. PMID:15375565

Coutelle C, Ward PJ, Fleury B *et al.* (1997). Laryngeal and oropharyngeal cancer, and alcohol dehydrogenase 3 and glutathione S-transferase M1 polymorphisms. *Hum Genet*, 99: 319–325. doi:10.1007/s004390050365 PMID:9050916

Couzigou P, Fleury B, Groppi A *et al.* (1991). Role of alcohol dehydrogenase polymorphism in ethanol metabolism and alcohol-related diseases. *Adv Exp Med Biol*, 284: 263–270. PMID:2053481

Covolo L, Gelatti U, Talamini R *et al.* (2005). Alcohol dehydrogenase 3, glutathione S-transferase M1 and T1 polymorphisms, alcohol consumption and hepatocellular carcinoma (Italy). *Cancer Causes Control*, 16: 831–838. doi:10.1007/s10552-005-2302-2 PMID:16132793

Crabb DW (1995). Ethanol oxidizing enzymes: roles in alcohol metabolism and alcoholic liver disease. *Prog Liver Dis*, 13: 151–172. PMID:9224501

Crabb DW (1997). First pass metabolism of ethanol: gastric or hepatic, mountain or molehill? *Hepatology*, 25: 1292–1294. doi:10.1002/hep.510250543 PMID:9141457

Crabb DW, Bosron WF, Li T-K (1983). Steady-state kinetic properties of purified rat liver alcohol dehydrogenase: application to predicting alcohol elimination rates in vivo. *Arch Biochem Biophys*, 224: 299–309. doi:10.1016/0003-9861(83)90213-8 PMID:6347067

Crabb DW, Edenberg HJ, Bosron WF, Li T-K (1989). Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive ALDH2(2) allele is dominant. *J Clin Invest*, 83: 314–316. doi:10.1172/JCI113875 PMID:2562960

Crabb DW & Liangpunsakul S (2007). Acetaldehyde generating enzyme systems: roles of alcohol dehydrogenase, CYP2E1 and catalase, and speculations on the role of other enzymes and processes. *Novartis Found Symp*, 285: 4–16, discussion 16–22, 198–199. doi:10.1002/9780470511848.ch2 PMID:17590984

Crebelli R, Conti G, Conti L, Carere A (1989). A comparative study on ethanol and acetaldehyde as inducers of chromosome malsegregation in Aspergillus nidulans. *Mutat Res*, 215: 187–195. PMID:2689879

Crosas B, Allali-Hassani A, Martínez SE *et al.* (2000). Molecular basis for differential substrate specificity in class IV alcohol dehydrogenases: a conserved function in retinoid metabolism but not in ethanol oxidation. *J Biol Chem*, 275: 25180–25187. doi:10.1074/jbc.M910040199 PMID:10829036

Crow KE, Braggins TJ, Batt RD, Hardman MJ (1982). Rat liver cytosolic malate dehydrogenase: purification, kinetic properties, role in control of free cytosolic NADH concentration. Analysis of control of ethanol metabolism using computer simulation. *J Biol Chem*, 257: 14217–14225. PMID:7142202

Cui Y, Morgenstern H, Greenland S *et al.* (2006). Polymorphism of Xeroderma pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. *Int J Cancer*, 118: 714–720. doi:10.1002/ijc.21413 PMID:16094634

Curtin K, Bigler J, Slattery ML *et al.* (2004). MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*, 13: 285–292. doi:10.1158/1055-9965.EPI-03-0083 PMID:14973104

Daiber A, Frein D, Namgaladze D, Ullrich V (2002). Oxidation and nitrosation in the nitrogen monoxide/superoxide system. *J Biol Chem*, 277: 11882–11888. doi:10.1074/jbc.M111988200 PMID:11805115

Daniel A & Roane D (1987). Aneuploidy is not induced by ethanol during spermatogenesis in the Chinese hamster. *Cytogenet Cell Genet*, 44: 43–48. doi:10.1159/000132339 PMID:3816304

Day NL, Richardson G, Robles N *et al.* (1990). Effect of prenatal alcohol exposure on growth and morphology of offspring at 8 months of age. *Pediatrics*, 85: 748–752. PMID:2330235

de Raat WK, Davis PB, Bakker GL (1983). Induction of sister-chromatid exchanges by alcohol and alcoholic beverages after metabolic activation by rat-liver homogenate. *Mutat Res*, 124: 85–90. doi:10.1016/0165-1218(83)90187-8 PMID:6633558

De Torok D (1972). Chromosomal irregularities in alcoholics. *Ann N Y Acad Sci*, 197: Suppl 190–100. doi:10.1111/j.1749-6632.1972.tb28125.x PMID:4504611

De Waziers I, Garlatti M, Bouguet J *et al.* (1995). Insulin down-regulates cytochrome P450 2B and 2E expression at the post-transcriptional level in the rat hepatoma cell line. *Mol Pharmacol*, 47: 474–479. PMID:7700245

Dees WL, Srivastava VK, Hiney JK (2001). Alcohol and female puberty: the role of intraovarian systems. *Alcohol Res Health*, 25: 271–275. PMID:11910704

Dey A & Cederbaum AI (2006). Alcohol and oxidative liver injury. *Hepatology*, 43: Suppl 1S63–S74. doi:10.1002/hep.20957 PMID:16447273

Díaz LE, Montero A, González-Gross M *et al.* (2002). Influence of alcohol consumption on immunological status: a review. *Eur J Clin Nutr*, 56: Suppl 3S50–S53. doi:10.1038/sj.ejcn.1601486 PMID:12142963

Díaz Gómez MI, Castro GD, de Layño AM *et al.* (2000). Cytochrome P450 reductase-mediated anaerobic biotransformation of ethanol to 1-hydroxyethyl-free radicals and acetaldehyde. *Toxicology*, 154: 113–122. doi:10.1016/S0300-483X(00)00325-5 PMID:11118675

Diczfalusy MA, Björkhem I, Einarsson C *et al.* (2001). Characterization of enzymes involved in formation of ethyl esters of long-chain fatty acids in humans. *J Lipid Res*, 42: 1025–1032. PMID:11441128

Dilger K, Metzler J, Bode JC, Klotz U (1997). CYP2E1 activity in patients with alcoholic liver disease. *J Hepatol*, 27: 1009–1014. doi:10.1016/S0168-8278(97)80144-4 PMID:9453426

Dinda PK, Kossev P, Beck IT, Buell MG (1996). Role of xanthine oxidase-derived oxidants and leukocytes in ethanol-induced jejunal mucosal injury. *Dig Dis Sci*, 41: 2461–2470. doi:10.1007/BF02100144 PMID:9011459

DiPadova C, Worner TM, Julkunen RJK, Lieber CS (1987). Effects of fasting and chronic alcohol consumption on the first-pass metabolism of ethanol. *Gastroenterology*, 92: 1169–1173. PMID:3557012

Dipple KM, Qulali M, Ross RA, Crabb DW (1993). Effects of thyroxine on the expression of alcohol dehydrogenase in rat liver and kidney. *Hepatology*, 17: 701–706. doi:10.1002/hep.1840170426 PMID:8477975

Djordjević D, Nikolić J, Stefanović V (1998). Ethanol interactions with other cytochrome P450 substrates including drugs, xenobiotics, and carcinogens. *Pathol Biol (Paris)*, 46: 760–770. PMID:9922992

Dohmen K, Baraona E, Ishibashi H *et al.* (1996). Ethnic differences in gastric sigma-alcohol dehydrogenase activity and ethanol first-pass metabolism. *Alcohol Clin Exp Res*, 20: 1569–1576. doi:10.1111/j.1530-0277.1996.tb01701.x PMID:8986205

Dong Y-J, Peng T-K, Yin S-J (1996). Expression and activities of class IV alcohol dehydrogenase and class III aldehyde dehydrogenase in human mouth. *Alcohol*, 13: 257–262. doi:10.1016/0741-8329(95)02052-7 PMID:8734840

Dorgan JF, Reichman ME, Judd JT *et al.* (1994). The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control*, 5: 53–60.. doi:10.1007/BF01830726 PMID:8123779

Dorgan JF, Baer DJ, Albert PS *et al.* (2001). Serum hormones and the alcohol–breast cancer association in postmenopausal women. *J Natl Cancer Inst*, 93: 710–715. doi:10.1093/jnci/93.9.710 PMID:11333294

Doyle KM, Bird DA, al-Salihi S *et al.* (1994). Fatty acid ethyl esters are present in human serum after ethanol ingestion. *J Lipid Res*, 35: 428–437. PMID:8014578

Du X, Squier CA, Kremer MJ, Wertz PW (2000). Penetration of N-nitrosonornicotine (NNN) across oral mucosa in the presence of ethanol and nicotine. *J Oral Pathol Med*, 29: 80–85. doi:10.1034/j.1600-0714.2000.290205.x PMID:10718403

Duester G, Farrés J, Felder MR *et al.* (1999). Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. *Biochem Pharmacol*, 58: 389–395. doi:10.1016/S0006-2952(99)00065-9 PMID:10424757

Dulout FN & Furnus CC (1988). Acetaldehyde-induced aneuploidy in cultured Chinese hamster cells. *Mutagenesis*, 3: 207–211. doi:10.1093/mutage/3.3.207 PMID:3045482

Dumitrescu RG & Shields PG (2005). The etiology of alcohol-induced breast cancer. *Alcohol*, 35: 213–225. doi:10.1016/j.alcohol.2005.04.005 PMID:16054983

Dunn SR, Simenhoff ML, Lele PS *et al.* (1990). N-Nitrosodimethylamine blood levels in patients with chronic renal failure: modulation of levels by ethanol and ascorbic acid. *J Natl Cancer Inst*, 82: 783–787. doi:10.1093/jnci/82.9.783 PMID:2325149

Edenberg HJ (2000). Regulation of the mammalian alcohol dehydrogenase genes. *Prog Nucleic Acid Res Mol Biol*, 64: 295–341. doi:10.1016/S0079-6603(00)64008-4 PMID:10697413

Edenberg HJ, Jerome RE, Li M (1999). Polymorphism of the human alcohol dehydrogenase 4 (ADH4) promoter affects gene expression. *Pharmacogenetics*, 9: 25–30. doi:10.1097/00008571-199902000-00004 PMID:10208639

Edenberg HJ, Xuei X, Chen H-J *et al.* (2006). Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. *Hum Mol Genet*, 15: 1539–1549. doi:10.1093/hmg/ddl073 PMID:16571603

Ehlers CL, Spence JP, Wall TL *et al.* (2004). Association of ALDH1 promoter polymorphisms with alcohol-related phenotypes in southwest California Indians. *Alcohol Clin Exp Res*, 28: 1481–1486. doi:10.1097/01.ALC.0000141821.06062.20 PMID:15597079

Ehrig T, Bosron WF, Li T-K (1990). Alcohol and aldehyde dehydrogenase. *Alcohol Alcohol*, 25: 105–116. PMID:2198030

Eker P & Sanner T (1986). Initiation of in vitro cell transformation by formaldehyde and acetaldehyde as measured by attachment-independent survival of cells in aggregates. *Eur J Cancer Clin Oncol*, 22: 671–676. doi:10.1016/0277-5379(86)90164-1 PMID:3743603

El-Rayes BF, Ali S, Heilbrun LK *et al.* (2003). Cytochrome p450 and glutathione transferase expression in human breast cancer. *Clin Cancer Res*, 9: 1705–1709. PMID:12738724

Elahi A, Zheng Z, Park J *et al.* (2002). The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. *Carcinogenesis*, 23: 1229–1234. doi:10.1093/carcin/23.7.1229 PMID:12117782

Emanuele MA & Emanuele NV (1998). Alcohol's effects on male reproduction. *Alcohol Health Res World*, 22: 195–201. PMID:15706796

Emanuele MA, Wezeman F, Emanuele NV (2002). Alcohol's effects on female reproductive function. *Alcohol Res Health*, 26: 274–281. PMID:12875037

Engeland K & Maret W (1993). Extrahepatic, differential expression of four classes of human alcohol dehydrogenase. *Biochem Biophys Res Commun*, 193: 47–53. doi:10.1006/bbrc.1993.1588 PMID:8503936

Enomoto N, Takase S, Takada N, Takada A (1991b). Alcoholic liver disease in heterozygotes of mutant and normal aldehyde dehydrogenase-2 genes. *Hepatology*, 13: 1071–1075. doi:10.1002/hep.1840130611 PMID:2050324

Enomoto N, Takase S, Yasuhara M, Takada A (1991a). Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. *Alcohol Clin Exp Res*, 15: 141–144. doi:10.1111/j.1530-0277.1991.tb00532.x PMID:2024727

Eriksson CJ, Fukunaga T, Lindman R (1994). Sex hormone response to alcohol. *Nature*, 369: 711 doi:10.1038/369711a0 PMID:8008063

Eriksson CJ, Widenius TV, Ylikahri RH *et al.* (1983). Inhibition of testosterone biosynthesis by ethanol. Relation to hepatic and testicular acetaldehyde, ketone bodies and cytosolic redox state in rats. *Biochem J*, 210: 29–36. PMID:6847648

Eriksson CJP, Fukunaga T, Sarkola T *et al.* (2001). Functional relevance of human adh polymorphism. *Alcohol Clin Exp Res*, 25: Suppl ISBRA157S–163S. PMID:11391066

Estonius M, Danielsson O, Karlsson C *et al.* (1993). Distribution of alcohol and sorbitol dehydrogenases. Assessment of mRNA species in mammalian tissues. *Eur J Biochem*, 215: 497–503. doi:10.1111/j.1432-1033.1993.tb18059.x PMID:8344317

Estonius M, Svensson S, Höög J-O (1996). Alcohol dehydrogenase in human tissues: localisation of transcripts coding for five classes of the enzyme. *FEBS Lett*, 397: 338–342. doi:10.1016/S0014-5793(96)01204-5 PMID:8955375

Eysseric H, Gonthier B, Soubeyran A *et al.* (2000). Effects of chronic ethanol exposure on acetaldehyde and free radical production by astrocytes in culture. *Alcohol*, 21: 117–125. doi:10.1016/S0741-8329(00)00075-6 PMID:10963934

Fang J-L & Vaca CE (1995). Development of a 32P-postlabelling method for the analysis of adducts arising through the reaction of acetaldehyde with 2′-deoxyguanosine-3′-monophosphate and DNA. *Carcinogenesis*, 16: 2177–2185. doi:10.1093/carcin/16.9.2177 PMID:7554072

Fang J-L & Vaca CE (1997). Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. *Carcinogenesis*, 18: 627–632. doi:10.1093/carcin/18.4.627 PMID:9111191

Farinati F, Lieber CS, Garro AJ (1989). Effects of chronic ethanol consumption on carcinogen activating and detoxifying systems in rat upper alimentary tract tissue. *Alcohol Clin Exp Res*, 13: 357–360. doi:10.1111/j.1530-0277.1989.tb00334.x PMID:2502039

Farinati F, Zhou Z, Bellah J *et al.* (1985). Effect of chronic ethanol consumption on activation of nitrosopyrrolidine to a mutagen by rat upper alimentary tract, lung, and hepatic tissue. *Drug Metab Dispos*, 13: 210–214. PMID:2859170

Farrés J, Moreno A, Crosas B *et al.* (1994b). Alcohol dehydrogenase of class IV (sigma sigma-ADH) from human stomach. cDNA sequence and structure/function relationships. *Eur J Biochem*, 224: 549–557. doi:10.1111/j.1432-1033.1994.00549.x PMID:7925371

Farrés J, Wang X, Takahashi K *et al.* (1994a). Effects of changing glutamate 487 to lysine in rat and human liver mitochondrial aldehyde dehydrogenase. A model to

study human (Oriental type) class 2 aldehyde dehydrogenase. *J Biol Chem*, 269: 13854–13860. PMID:7910607

Fataccioli V, Andraud E, Gentil M *et al.* (1999). Effects of chronic ethanol administration on rat liver proteasome activities: relationship with oxidative stress. *Hepatology*, 29: 14–20. doi:10.1002/hep.510290106 PMID:9862843

Feierman DE, Melinkov Z, Nanji AA (2003). Induction of CYP3A by ethanol in multiple in vitro and in vivo models. *Alcohol Clin Exp Res*, 27: 981–988. PMID:12824820

Fernandes PH, Kanuri M, Nechev LV *et al.* (2005). Mammalian cell mutagenesis of the DNA adducts of vinyl chloride and crotonaldehyde. *Environ Mol Mutagen*, 45: 455–459. doi:10.1002/em.20117 PMID:15690339

Fernández-Solà J, Fatjó F, Sacanella E *et al.* (2006). Evidence of apoptosis in alcoholic cardiomyopathy. *Hum Pathol*, 37: 1100–1110. doi:10.1016/j.humpath.2006.03.022 PMID:16867874

Fillmore KM, Kerr WC, Stockwell T *et al.* (2006). Moderate alcohol use and reduced mortality risk: Systematic error in prospestive studies. *Addict Res Theory*, 14: 101–132. doi:10.1080/16066350500497983

Fong WP, Cheng CH, Tang WK (2006). Antiquitin, a relatively unexplored member in the superfamily of aldehyde dehydrogenases with diversified physiological functions. *Cell Mol Life Sci*, 63: 2881–2885. doi:10.1007/s00018-006-6089-4 PMID:17131062

Frank J, Witte K, Schrödl W, Schütt C (2004). Chronic alcoholism causes deleterious conditioning of innate immunity. *Alcohol Alcohol*, 39: 386–392. PMID:15289211

Fraser AG (1997). Pharmacokinetic interactions between alcohol and other drugs. *Clin Pharmacokinet*, 33: 79–90. doi:10.2165/00003088-199733020-00001 PMID:9260032

French SW (2005). The pathogenesis and significance of the urinary alcohol cycle in rats fed ethanol intragastrically. *Alcohol Clin Exp Res*, 29: Suppl 11158S–161S. doi:10.1097/01.alc.0000189282.91730.c8 PMID:16344601

French SW, Wong K, Jui L *et al.* (1993). Effect of ethanol on cytochrome P450 2E1 (CYP2E1), lipid peroxidation, and serum protein adduct formation in relation to liver pathology pathogenesis. *Exp Mol Pathol*, 58: 61–75. doi:10.1006/exmp.1993.1006 PMID:8454037

Freudenheim JL, Ambrosone CB, Moysich KB *et al.* (1999). Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk. *Cancer Causes Control*, 10: 369–377. doi:10.1023/A:1008950717205 PMID:10530606

Frezza M, di Padova C, Pozzato G *et al.* (1990). High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. *N Engl J Med*, 322: 95–99. doi:10.1056/NEJM199001113220205 PMID:2248624

Friso S, Choi S-W, Girelli D *et al.* (2002). A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an

interaction with folate status. *Proc Natl Acad Sci U S A*, 99: 5606–5611. doi:10.1073/pnas.062066299 PMID:11929966

Fritsche E, Pittman GS, Bell DA (2000). Localization, sequence analysis, and ethnic distribution of a 96-bp insertion in the promoter of the human CYP2E1 gene. *Mutat Res*, 432: 1–5. PMID:10729706

Frosst P, Blom HJ, Milos R *et al.* (1995). A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*, 10: 111–113. doi:10.1038/ng0595-111 PMID:7647779

Fujimiya T, Yamaoka K, Fukui Y (1989). Parallel first-order and Michaelis-Menten elimination kinetics of ethanol. Respective role of alcohol dehydrogenase (ADH), non-ADH and first-order pathways. *J Pharmacol Exp Ther*, 249: 311–317. PMID:2709333

Fukushima M, Suzuki H, Seino Y (2004). Insulin secretion capacity in the development from normal glucose tolerance to type 2 diabetes. *Diabetes Res Clin Pract*, 66: Suppl 1S37–S43. doi:10.1016/j.diabres.2003.11.024 PMID:15563978

Gao C, Takezaki T, Wu J *et al.* (2002). Interaction between cytochrome P-450 2E1 polymorphisms and environmental factors with risk of esophageal and stomach cancers in Chinese. *Cancer Epidemiol Biomarkers Prev*, 11: 29–34. PMID:11815398

Garro AJ, Espina N, Farinati F, Salvagnini M (1986). The effects of chronic ethanol consumption on carcinogen metabolism and on O6-methylguanine transferase-mediated repair of alkylated DNA. *Alcohol Clin Exp Res*, 10: Suppl73S–77S. doi:10.1111/j.1530-0277.1986.tb05184.x PMID:3544934

Garte S, Gaspari L, Alexandrie A-K *et al.* (2001). Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev*, 10: 1239–1248. PMID:11751440

Gattás GJ & Saldanha PH (1997). Chromosomal aberrations in peripheral lymphocytes of abstinent alcoholics. *Alcohol Clin Exp Res*, 21: 238–243. doi:10.1111/j.1530-0277.1997.tb03755.x PMID:9113258

Gattás GJF, de Carvalho MB, Siraque MS *et al.* (2006). Genetic polymorphisms of CYP1A1, CYP2E1, GSTM1, and GSTT1 associated with head and neck cancer. *Head Neck*, 28: 819–826. doi:10.1002/hed.20410 PMID:16721740

Gavaler JS (1995). Alcohol effects on hormone levels in normal postmenopausal women and in postmenopausal women with alcohol-induced cirrhosis. *Recent Dev Alcohol*, 12: 199–208. doi:10.1007/0-306-47138-8_11 PMID:7624541

Gavaler JS (1998). Alcoholic beverages as a source of estrogens. *Alcohol Health Res World*, 22: 220–227. PMID:15706799

Gemma S, Vichi S, Testai E (2006). Individual susceptibility and alcohol effects:biochemical and genetic aspects. *Ann Ist Super Sanita*, 42: 8–16. PMID:16801720

Ghanayem BI (2007). Inhibition of urethane-induced carcinogenicity in cyp2e1-/- in comparison to cyp2e1+/+ mice. *Toxicol Sci*, 95: 331–339. doi:10.1093/toxsci/kfl158 PMID:17093202

Gibel W (1967). Experimental studies of syncarcinogenesis in esophageal carcinoma *Arch Geschwulstforsch*, 30: 181–189. PMID:4299442

Ginsburg ES, Mello NK, Mendelson JH *et al.* (1996). Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA*, 276: 1747–1751. doi:10.1001/jama.276.21.1747 PMID:8940324

Giovannucci E (2004). Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. *J Nutr*, 134: 2475S–2481S. PMID:15333745

Giovannucci E, Chen J, Smith-Warner SA *et al.* (2003). Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev*, 12: 970–979. PMID:14578131

Girnun GD, Domann FE, Moore SA, Robbins MEC (2002). Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter. *Mol Endocrinol*, 16: 2793–2801. doi:10.1210/me.2002-0020 PMID:12456800

Goedde HW, Agarwal DP, Fritze G *et al.* (1992). Distribution of ADH2 and ALDH2 genotypes in different populations. *Hum Genet*, 88: 344–346. doi:10.1007/BF00197271 PMID:1733836

Goedde HW, Harada S, Agarwal DPC (1979). Racial differences in alcohol sensitivity: a new hypothesis. *Hum Genet*, 51: 331–334. doi:10.1007/BF00283404 PMID:511165

Goist KC Jr & Sutker PB (1985). Acute alcohol intoxication and body composition in women and men. *Pharmacol Biochem Behav*, 22: 811–814. doi:10.1016/0091-3057(85)90532-5 PMID:4011640

Gonzalez FJ (2007). The 2006 Bernard B. Brodie award lecture: CYP2E1. *Drug Metab Dispos*, 35: 1–8. doi:10.1124/dmd.106.012492 PMID:17020953

González MV, Alvarez V, Pello MF *et al.* (1998). Genetic polymorphism of N-acetyltransferase-2, glutathione S-transferase-M1, and cytochromes P450IIE1 and P450IID6 in the susceptibility to head and neck cancer. *J Clin Pathol*, 51: 294–298. doi:10.1136/jcp.51.4.294 PMID:9659241

Gonzalez-Quintela A, Vidal C, Gude F (2004). Alcohol, IgE and allergy. *Addict Biol*, 9: 195–204. doi:10.1080/13556210412331292235 PMID:15511713

Goode EL, Potter JD, Bigler J, Ulrich CM (2004). Methionine synthase D919G polymorphism, folate metabolism, and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev*, 13: 157–162. doi:10.1158/1055-9965.EPI-03-0097 PMID:14744749

Goodlett CR, Horn KH, Zhou FC (2005). Alcohol teratogenesis: mechanisms of damage and strategies for intervention. *Exp Biol Med (Maywood)*, 230: 394–406. PMID:15956769

Graf U, Moraga AA, Castro R, Díaz Carrillo E (1994). Genotoxicity testing of different types of beverages in the Drosophila wing Somatic Mutation And Recombination Test. *Food Chem Toxicol*, 32: 423–430. doi:10.1016/0278-6915(94)90040-X PMID:8206441

Grattagliano I, Palmieri V, Vendemiale G *et al.* (1999). Chronic ethanol administration induces oxidative alterations and functional impairment of pancreatic mitochondria in the rat. *Digestion*, 60: 549–553. doi:10.1159/000007705 PMID:10545725

Griciute L, Castegnaro M, Béréziat J-C (1981). Influence of ethyl alcohol on carcinogenesis with N-nitrosodimethylamine. *Cancer Lett*, 13: 345–352. doi:10.1016/0304-3835(81)90063-X PMID:7306961

Griciute L, Castegnaro M, Béréziat J-C (1982) Influence of ethyl alcohol on the carcinogenic activity of *N*-nitrosodi-*n*-propylamine. In: Bartsch, H., O'Neill, I. K., Castegnaro, M. & Okada, M., ed., N-*Nitroso Compounds: Occurrence and Biological Effects. IARC Scientific Publications, No. 41*, Lyon, International Agency for Research on Cancer, pp. 643–648. –

Griciute L, Castegnaro M, Béréziat JC (1987) Influence of ethyl alcohol on carcinogenesis induced by volatile N-nitrosamines detected in alcoholic beverages. In: Bartsch, H., O'Neill, I. K., & Schulte-Hermann, R., ed., *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms. IARC Scientific Publications, No. 84*, Lyon, International Agency for Research on Cancer, pp. 264–265.

Griciute L, Castegnaro M, Béréziat J-C, Cabral JRP (1986). Influence of ethyl alcohol on the carcinogenic activity of N-nitrosonornicotine. *Cancer Lett*, 31: 267–275. doi:10.1016/0304-3835(86)90147-3 PMID:3719567

Gubała W & Zuba D (2002). Saliva as an alternative specimen for alcohol determination in the human body. *Pol J Pharmacol*, 54: 161–165. PMID:12139114

Guengerich FP, Shimada T, Yun C-H *et al.* (1994). Interactions of ingested food, beverage, and tobacco components involving human cytochrome P4501A2, 2A6, 2E1, and 3A4 enzymes. *Environ Health Perspect*, 102: Suppl 949–53. PMID:7698084

Gullo L (2005). Alcohol and chronic pancreatitis: leading or secondary etiopathogenetic role? *JOP*, 6: Suppl 168–72. PMID:15650289

Gunzerath L, Faden V, Zakhari S, Warren K (2004). National Institute on Alcohol Abuse and Alcoholism report on moderate drinking. *Alcohol Clin Exp Res*, 28: 829–847. PMID:15201626

Haber PS, Apte MV, Applegate TL *et al.* (1998). Metabolism of ethanol by rat pancreatic acinar cells. *J Lab Clin Med*, 132: 294–302. doi:10.1016/S0022-2143(98)90042-7 PMID:9794700

Habs M & Schmähl D (1981). Inhibition of the hepatocarcinogenic activity of diethylnitrosamine (DENA) by ethanol in rats. *Hepatogastroenterology*, 28: 242–244. PMID:7345007

Haddad JJ (2004). Alcoholism and neuro-immune-endocrine interactions: physiochemical aspects. *Biochem Biophys Res Commun*, 323: 361–371. doi:10.1016/j.bbrc.2004.08.119 PMID:15369760

Haddock NF & Wilkin JK (1982). Cutaneous reactions to lower aliphatic alcohols before and during disulfiram therapy. *Arch Dermatol*, 118: 157–159. doi:10.1001/archderm.118.3.157 PMID:7065662

Hakkak R, Korourian S, Ronis MJJ *et al.* (1996). Effects of diet and ethanol on the expression and localization of cytochromes P450 2E1 and P450 2C7 in the colon of male rats. *Biochem Pharmacol*, 51: 61–69. doi:10.1016/0006-2952(95)02154-X PMID:8534269

Halsted CH, Robles EA, Mezey E (1973). Distribution of ethanol in the human gastrointestinal tract. *Am J Clin Nutr*, 26: 831–834. PMID:4720670

Halsted CH, Villanueva JA, Devlin AM, Chandler CJ (2002). Metabolic interactions of alcohol and folate. *J Nutr*, 132: Suppl2367S–2372S. PMID:12163694

Hamitouche S, Poupon J, Dreano Y *et al.* (2006). Ethanol oxidation into acetaldehyde by 16 recombinant human cytochrome P450 isoforms: role of CYP2C isoforms in human liver microsomes. *Toxicol Lett*, 167: 221–230. doi:10.1016/j.toxlet.2006.09.011 PMID:17084997

Han J, Tranah GJ, Hankinson SE *et al.* (2006). Polymorphisms in O6-methylguanine DNA methyltransferase and breast cancer risk. *Pharmacogenet Genomics*, 16: 469–474. doi:10.1097/01.fpc.0000215065.21718.4c PMID:16788379

Harada S, Agarwal DP, Goedde HW (1981). Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. *Lancet*, 2: 982 doi:10.1016/S0140-6736(81)91172-7 PMID:6117742

Harada S, Agarwal DP, Goedde HW (1985). Aldehyde dehydrogenase polymorphism and alcohol metabolism in alcoholics. *Alcohol*, 2: 391–392. doi:10.1016/0741-8329(85)90100-4 PMID:4026955

Harada S, Agarwal DP, Goedde HW *et al.* (1982). Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet*, 2: 827 doi:10.1016/S0140-6736(82)92722-2 PMID:6126701

Harada S, Agarwal DP, Goedde HW, Ishikawa B (1983). Aldehyde dehydrogenase isozyme variation and alcoholism in Japan. *Pharmacol Biochem Behav*, 18: Suppl 1151–153. doi:10.1016/0091-3057(83)90163-6 PMID:6634831

Harada S, Okubo T, Nakamura T *et al.* (1999). A novel polymorphism (-357 G/A) of the ALDH2 gene: linkage disequilibrium and an association with alcoholism. *Alcohol Clin Exp Res*, 23: 958–962. doi:10.1111/j.1530-0277.1999.tb04212.x PMID:10397278

Harty LC, Caporaso NE, Hayes RB *et al.* (1997). Alcohol dehydrogenase 3 genotype and risk of oral cavity and pharyngeal cancers. *J Natl Cancer Inst*, 89: 1698–1705. doi:10.1093/jnci/89.22.1698 PMID:9390539

Haseba T, Duester G, Shimizu A *et al.* (2006). In vivo contribution of Class III alcohol dehydrogenase (ADH3) to alcohol metabolism through activation by cytoplasmic solution hydrophobicity. *Biochim Biophys Acta*, 1762: 276–283. PMID:16431092

Hashibe M, Boffetta P, Zaridze D *et al.* (2006). Evidence for an important role of alcohol- and aldehyde-metabolizing genes in cancers of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev*, 15: 696–703. doi:10.1158/1055-9965.EPI-05-0710 PMID:16614111

Hashimoto T, Uchida K, Okayama N *et al.* (2006). ALDH2 1510 G/A (Glu487Lys) polymorphism interaction with age in head and neck squamous cell carcinoma. *Tumour Biol*, 27: 334–338. doi:10.1159/000096126 PMID:17033202

Hasin D, Aharonovich E, Liu X *et al.* (2002). Alcohol dependence symptoms and alcohol dehydrogenase 2 polymorphism: Israeli Ashkenazis, Sephardics, and recent Russian immigrants. *Alcohol Clin Exp Res*, 26: 1315–1321. PMID:12351924

Hayashi S-I, Watanabe J, Kawajiri K (1991). Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *J Biochem*, 110: 559–565. PMID:1778977

He L, Ronis MJJ, Badger TM (2002). Ethanol induction of class I alcohol dehydrogenase expression in the rat occurs through alterations in CCAAT/enhancer binding proteins β and γ. *J Biol Chem*, 277: 43572–43577. doi:10.1074/jbc.M204535200 PMID:12213809

He L, Simmen FA, Ronis MJJ, Badger TM (2004). Post-transcriptional regulation of sterol regulatory element-binding protein-1 by ethanol induces class I alcohol dehydrogenase in rat liver. *J Biol Chem*, 279: 28113–28121. doi:10.1074/jbc.M400906200 PMID:15123720

He S-M & Lambert B (1985). Induction and persistence of SCE-inducing damage in human lymphocytes exposed to vinyl acetate and acetaldehyde in vitro. *Mutat Res*, 158: 201–208. doi:10.1016/0165-1218(85)90086-2 PMID:4079951

He S-M & Lambert B (1990). Acetaldehyde-induced mutation at the *HPRT* locus in human lymphocytes in vitro. *Environ Mol Mutagen*, 16: 57–63. doi:10.1002/em.2850160202 PMID:2209564

Helander A & Lindahl-Kiessling K (1991). Increased frequency of acetaldehyde-induced sister-chromatid exchanges in human lymphocytes treated with an aldehyde dehydrogenase inhibitor. *Mutat Res*, 264: 103–107. doi:10.1016/0165-7992(91)90124-M PMID:1944390

Hellmér L & Bolcsfoldi G (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. *Mutat Res*, 272: 145–160. PMID:1383747

Hillbom ME, Lindros KO, Larsen A (1981). The calcium carbimide-ethanol interaction: lack of relation between electroencephalographic response and cerebrospinal fluid acetaldehyde. *Toxicol Lett*, 9: 113–119. doi:10.1016/0378-4274(81)90026-6 PMID:7302982

Hines LM, Hankinson SE, Smith-Warner SA *et al.* (2000). A prospective study of the effect of alcohol consumption and ADH3 genotype on plasma steroid hormone levels and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, 9: 1099–1105. PMID:11045794

Hirano T, Kaplowitz N, Tsukamoto H *et al.* (1992). Hepatic mitochondrial glutathione depletion and progression of experimental alcoholic liver disease in rats. *Hepatology*, 16: 1423–1427. doi:10.1002/hep.1840160619 PMID:1446896

Hirose M, Kono S, Tabata S *et al.* (2005). Genetic polymorphisms of methylenetetrahydrofolate reductase and aldehyde dehydrogenase 2, alcohol use and risk of colorectal adenomas: Self-Defense Forces Health Study. *Cancer Sci*, 96: 513–518. doi:10.1111/j.1349-7006.2005.00077.x PMID:16108833

Hoek JB, Cahill A, Pastorino JG (2002). Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology*, 122: 2049–2063. doi:10.1053/gast.2002.33613 PMID:12055609

Hoffler U & Ghanayem BI (2005). Increased bioaccumulation of urethane in CYP2E1-/- versus CYP2E1+/+ mice. *Drug Metab Dispos*, 33: 1144–1150. doi:10.1124/dmd.105.003806 PMID:15879495

Holford NHG (1987). Clinical pharmacokinetics of ethanol. *Clin Pharmacokinet*, 13: 273–292. doi:10.2165/00003088-198713050-00001 PMID:3319346

Holt S (1981). Observations on the relation between alcohol absorption and the rate of gastric emptying. *Can Med Assoc J*, 124: 267–277, 297. PMID:7459787

Homann N, Jousimies-Somer H, Jokelainen K *et al.* (1997). High acetaldehyde levels in saliva after ethanol consumption: methodological aspects and pathogenetic implications. *Carcinogenesis*, 18: 1739–1743. doi:10.1093/carcin/18.9.1739 PMID:9328169

Homann N, Stickel F, König IR *et al.* (2006). Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. *Int J Cancer*, 118: 1998–2002. doi:10.1002/ijc.21583 PMID:16287084

Homann N, Tillonen J, Salaspuro M (2000b). Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. *Int J Cancer*, 86: 169–173. doi:10.1002/(SICI)1097-0215(20000415)86:2<169::AID-IJC4>3.0.CO;2-3 PMID:10738242

Homann N, Tillonen JH, Meurman JH *et al.* (2000a). Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. *Carcinogenesis*, 21: 663–668. doi:10.1093/carcin/21.4.663 PMID:10753201

Hong Y-C, Lee K-H, Kim W-C *et al.* (2005). Polymorphisms of XRCC1 gene, alcohol consumption and colorectal cancer. *Int J Cancer*, 116: 428–432. doi:10.1002/ijc.21019 PMID:15800946

Höög J-O & Brandt M (1995). Mammalian class VI alcohol dehydrogenase. Novel types of the rodent enzymes. *Adv Exp Med Biol*, 372: 355–364. PMID:7484399

Hori H, Kawano T, Endo M, Yuasa Y (1997). Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and human esophageal squamous cell carcinoma susceptibility. *J Clin Gastroenterol*, 25: 568–575. doi:10.1097/00004836-199712000-00003 PMID:9451664

Horie N, Aiba H, Oguro K *et al.* (1995). Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. *Cell Struct Funct*, 20: 191–197. doi:10.1247/csf.20.191 PMID:7586009

Hsu LC, Bendel RE, Yoshida A (1988). Genomic structure of the human mitochondrial aldehyde dehydrogenase gene. *Genomics*, 2: 57–65. doi:10.1016/0888-7543(88)90109-7 PMID:2838413

Hsu LC & Chang W-C (1991). Cloning and characterization of a new functional human aldehyde dehydrogenase gene. *J Biol Chem*, 266: 12257–12265. PMID:2061311

Hsu LC, Chang W-C, Lin SW, Yoshida A (1995). Cloning and characterization of genes encoding four additional human aldehyde dehydrogenase isozymes. *Adv Exp Med Biol*, 372: 159–168. PMID:7484374

Hsu LC, Chang W-C, Yoshida A (1989). Genomic structure of the human cytosolic aldehyde dehydrogenase gene. *Genomics*, 5: 857–865. doi:10.1016/0888-7543(89)90127-4 PMID:2591967

Hsu TC, Furlong C, Spitz MR (1991). Ethyl alcohol as a cocarcinogen with special reference to the aerodigestive tract: a cytogenetic study. *Anticancer Res*, 11: 1097–1101. PMID:1716084

Huang WY, Olshan AF, Schwartz SM *et al.* (2005). Selected genetic polymorphisms in MGMT, XRCC1, XPD, and XRCC3 and risk of head and neck cancer: a pooled analysis. *Cancer Epidemiol Biomarkers Prev*, 14: 1747–1753. doi:10.1158/1055-9965.EPI-05-0162 PMID:16030112

Huang Y-S, Chern H-D, Su W-J *et al.* (2003). Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. *Hepatology*, 37: 924–930. doi:10.1053/jhep.2003.50144 PMID:12668988

Hugues FC, Julien D, Bors V *et al.* (1980). Determination in man of the beta blocking properties and the pharmacological half of pargolol (Author's transl) *Therapie*, 35: 475–481. PMID:6110251

Hung H-C, Chuang J, Chien Y-C *et al.* (1997). Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev*, 6: 901–905. PMID:9367063

Hüttner E, Matthies U, Nikolova T, Ehrenreich H (1999). A follow-up study on chromosomal aberrations in lymphocytes of alcoholics during early, medium, and long-term abstinence. *Alcohol Clin Exp Res*, 23: 344–348. doi:10.1111/j.1530-0277.1999.tb04120.x PMID:10069566

IARC. (1985). Allyl Compounds, Aldehydes, Epoxides and Peroxides. *IARC Monogr Eval Carcinog Risk Chem Hum*, 36: 1–369.

IARC. (1988). Alcohol Drinking. *IARC Monogr Eval Carcinog Risks Hum*, 44: 1–378. PMID:3236394

IARC. (1999). Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, France, 17–24 February 1998. *IARC Monogr Eval Carcinog Risks Hum*, 71: 1–315. PMID:10507919

IARC. (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*, 82: 1–556. PMID:12687954

Iarmarcovai G, Bonassi S, Sari-Minodier I *et al.* (2007). Exposure to genotoxic agents, host factors, and lifestyle influence the number of centromeric signals in micronuclei: a pooled re-analysis. *Mutat Res*, 615: 18–27. PMID:17198715

Ingelman-Sundberg M, Ronis MJJ, Lindros KO *et al.* (1994). Ethanol-inducible cytochrome P4502E1: regulation, enzymology and molecular biology. *Alcohol Alcohol,*, Suppl 2131–139. PMID:8974327

Inoue K, Fukunaga M, Kiriyama T, Komura S (1984). Accumulation of acetaldehyde in alcohol-sensitive Japanese: relation to ethanol and acetaldehyde oxidizing capacity. *Alcohol Clin Exp Res*, 8: 319–322. doi:10.1111/j.1530-0277.1984.tb05519.x PMID:6377951

IPCS (1995) Environmental Health Criteria 167, Acetaldehyde. International Programme on Chemical Safety, Geneva, World Health Organization

Iranmanesh A, Veldhuis JD, Samojlik E *et al.* (1988). Alterations in the pulsatile properties of gonadotropin secretion in alcoholic men. *J Androl*, 9: 207–214. PMID:3136120

Ishikawa H, Miyatsu Y, Kurihara K, Yokoyama K (2006). Gene-environmental interactions between alcohol-drinking behavior and ALDH2 and CYP2E1 polymorphisms and their impact on micronuclei frequency in human lymphocytes. *Mutat Res*, 594: 1–9. PMID:16126235

Ishikawa H, Yamamoto H, Tian Y *et al.* (2003). Effects of ALDH2 gene polymorphisms and alcohol-drinking behavior on micronuclei frequency in non-smokers. *Mutat Res*, 541: 71–80. PMID:14568296

Isse T, Matsuno K, Oyama T *et al.* (2005). 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*, 29: 1959–1964. doi:10.1097/01.alc.0000187161.07820.21 PMID:16340452

Isse T, Oyama T, Kitagawa K *et al.* (2002). Diminished alcohol preference in transgenic mice lacking aldehyde dehydrogenase activity. *Pharmacogenetics*, 12: 621–626. doi:10.1097/00008571-200211000-00006 PMID:12439222

Itoga S, Nomura F, Makino Y *et al.* (2002). Tandem repeat polymorphism of the CYP2E1 gene: an association study with esophageal cancer and lung cancer. *Alcohol Clin Exp Res*, 26: Suppl15S–19S. PMID:12198369

Izzotti A, Balansky RM, Blagoeva PM *et al.* (1998). DNA alterations in rat organs after chronic exposure to cigarette smoke and/or ethanol ingestion. *FASEB J*, 12: 753–758. PMID:9619454

Jalas JR, Hecht SS, Murphy SE (2005). Cytochrome P450 enzymes as catalysts of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Chem Res Toxicol*, 18: 95–110. doi:10.1021/tx049847p PMID:15720112

Jamal MM, Saadi Z, Morgan TR (2005). Alcohol and hepatitis C. *Dig Dis*, 23: 285–296. doi:10.1159/000090176 PMID:16508293

Jansson T (1982). The frequency of sister chromatid exchanges in human lymphocytes treated with ethanol and acetaldehyde. *Hereditas*, 97: 301–303. PMID:7161122

JETOC (1997) *Mutagenicity Test Data of Existing Chemical Substances*, Supplement, Tokyo, Japanese Chemical Industry Ecology–Toxicology and Information Center, p. 94.

Jiang Z, Akey JM, Shi J *et al.* (2001). A polymorphism in the promoter region of catalase is associated with blood pressure levels. *Hum Genet*, 109: 95–98. doi:10.1007/s004390100553 PMID:11479740

Johansson I, Ekström G, Scholte B *et al.* (1988). Ethanol-, fasting-, and acetone-inducible cytochromes P-450 in rat liver: regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry*, 27: 1925–1934. doi:10.1021/bi00406a019 PMID:3378038

Jokelainen K, Matysiak-Budnik T, Mäkisalo H *et al.* (1996). High intracolonic acetaldehyde values produced by a bacteriocolonic pathway for ethanol oxidation in piglets. *Gut*, 39: 100–104. doi:10.1136/gut.39.1.100 PMID:8881818

Jones AW & Andersson L (1996). Influence of age, gender, and blood-alcohol concentration on the disappearance rate of alcohol from blood in drinking drivers. *J Forensic Sci*, 41: 922–926. PMID:8914281

Julià P, Farrés J, Parés X (1987). Characterization of three isoenzymes of rat alcohol dehydrogenase. Tissue distribution and physical and enzymatic properties. *Eur J Biochem*, 162: 179–189. doi:10.1111/j.1432-1033.1987.tb10559.x PMID:3816781

Julkunen RJK, Di Padova C, Lieber CS (1985). First pass metabolism of ethanol–A gastrointestinal barrier against the systemic toxicity of ethanol. *Life Sci*, 37: 567–573. doi:10.1016/0024-3205(85)90470-9 PMID:4021730

Justenhoven C, Hamann U, Pierl CB *et al.* (2005). One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. *Cancer Epidemiol Biomarkers Prev*, 14: 3015–3018. doi:10.1158/1055-9965.EPI-05-0592 PMID:16365030

Kamdem LK, Meineke I, Gödtel-Armbrust U *et al.* (2006). Dominant contribution of P450 3A4 to the hepatic carcinogenic activation of aflatoxin B1. *Chem Res Toxicol*, 19: 577–586. doi:10.1021/tx050358e PMID:16608170

Kaphalia BS, Fritz RR, Ansari GAS (1997). Purification and characterization of rat liver microsomal fatty acid ethyl and 2-chloroethyl ester synthase and their relationship with carboxylesterase (pI 6.1). *Chem Res Toxicol*, 10: 211–218. doi:10.1021/tx960079e PMID:9049433

Karaoğuz MY, Coşar B, Arikan Z *et al.* (2005). Increased frequency of sister chromatid exchanges in peripheral lymphocytes of alcoholics and cigarette smokers. *Cell Biol Int*, 29: 165–168. doi:10.1016/j.cellbi.2004.11.019 PMID:15774315

Kato S, Tajiri T, Matsukura N *et al.* (2003). Genetic polymorphisms of aldehyde dehydrogenase 2, cytochrome p450 2E1 for liver cancer risk in HCV antibody-positive Japanese patients and the variations of CYP2E1 mRNA expression levels in the liver due to its polymorphism. *Scand J Gastroenterol*, 38: 886–893. doi:10.1080/00365520310004489 PMID:12940444

Katoh T, Kaneko S, Kohshi K *et al.* (1999). Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. *Int J Cancer*, 83: 606–609. doi:10.1002/(SICI)1097-0215(19991126)83:5<606::AID-IJC6>3.0.CO;2-P PMID:10521794

Kedishvili NY, Gough WH, Chernoff EA *et al.* (1997). cDNA sequence and catalytic properties of a chick embryo alcohol dehydrogenase that oxidizes retinol and 3beta,5alpha-hydroxysteroids. *J Biol Chem*, 272: 7494–7500. doi:10.1074/jbc.272.11.7494 PMID:9054452

Kedishvili NY, Popov KM, Rougraff PM *et al.* (1992). CoA-dependent methylmalonate-semialdehyde dehydrogenase, a unique member of the aldehyde dehydrogenase superfamily. cDNA cloning, evolutionary relationships, and tissue distribution. *J Biol Chem*, 267: 19724–19729. PMID:1527093

Keku T, Millikan R, Worley K *et al.* (2002). 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. *Cancer Epidemiol Biomarkers Prev*, 11: 1611–1621. PMID:12496052

Kessova IG, DeCarli LM, Lieber CS (1998). Inducibility of cytochromes P-4502E1 and P-4501A1 in the rat pancreas. *Alcohol Clin Exp Res*, 22: 501–504. PMID:9581659

Kietthubthew S, Sriplung H, Au WW, Ishida T (2006). Polymorphism in DNA repair genes and oral squamous cell carcinoma in Thailand. *Int J Hyg Environ Health*, 209: 21–29. doi:10.1016/j.ijheh.2005.06.002 PMID:16373199

Kim RB, Yamazaki H, Chiba K *et al.* (1996). In vivo and in vitro characterization of CYP2E1 activity in Japanese and Caucasians. *J Pharmacol Exp Ther*, 279: 4–11. PMID:8858968

Klatsky AL (2002). Alcohol and cardiovascular diseases: a historical overview. *Ann N Y Acad Sci*, 957: 7–15. doi:10.1111/j.1749-6632.2002.tb02901.x PMID:12074957

Klotz U & Ammon E (1998). Clinical and toxicological consequences of the inductive potential of ethanol. *Eur J Clin Pharmacol*, 54: 7–12. doi:10.1007/s002280050412 PMID:9591923

Knadle S (1985). Synergistic interaction between hydroquinone and acetaldehyde in the induction of sister chromatid exchange in human lymphocytes in vitro. *Cancer Res*, 45: 4853–4857. PMID:4027972

Kohno T, Shinmura K, Tosaka M *et al.* (1998). Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene*, 16: 3219–3225. doi:10.1038/sj.onc.1201872 PMID:9681819

Koide T, Ohno T, Huang X-E *et al.* (2000). HBV/HCV infection, alcohol, tobacco and genetic polymorphisms for hepatocellular carcinoma in Nagoya, Japan. *Asian Pac J Cancer Prev*, 1: 237–243. PMID:12718671

Koivisto T, Carr LG, Li T-K, Eriksson CJ (1993). Mitochondrial aldehyde dehydrogenase (ALDH2) polymorphism in AA and ANA rats: lack of genotype and phenotype line differences. *Pharmacol Biochem Behav*, 45: 215–220. doi:10.1016/0091-3057(93)90107-5 PMID:8516360

Koko V, Todorović V, Nikolić JA *et al.* (1995). Rat pancreatic B-cells after chronic alcohol feeding. A morphometric and fine structural study. *Histol Histopathol*, 10: 325–337. PMID:7599431

Konishi N, Kitahori Y, Shimoyama T *et al.* (1986). Effects of sodium chloride and alcohol on experimental esophageal carcinogenesis induced by N-nitrosopiperidine in rats. *Jpn J Cancer Res*, 77: 446–451. PMID:3089977

Kono H, Bradford BU, Yin M *et al.* (1999). CYP2E1 is not involved in early alcohol-induced liver injury. *Am J Physiol*, 277: G1259–G1267. PMID:10600824

Kono H, Nakagami M, Rusyn I *et al.* (2001). Development of an animal model of chronic alcohol-induced pancreatitis in the rat. *Am J Physiol Gastrointest Liver Physiol*, 280: G1178–G1186. PMID:11352811

Kono S & Chen K (2005). Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. *Cancer Sci*, 96: 535–542. doi:10.1111/j.1349-7006.2005.00090.x PMID:16128738

Koppes LL, Dekker JM, Hendriks HF *et al.* (2005). Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care*, 28: 719–725. doi:10.2337/diacare.28.3.719 PMID:15735217

Kopun M & Propping P (1977). The kinetics of ethanol absorption and elimination in twins and supplementary repetitive experiments in singleton subjects. *Eur J Clin Pharmacol*, 11: 337–344. doi:10.1007/BF00566530 PMID:560303

Korte A, Obe G, Ingwersen I, Rückert G (1981). Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. *Mutat Res*, 88: 389–395. doi:10.1016/0165-1218(81)90030-6 PMID:7195980

Kostrubsky VE, Strom SC, Wood SG *et al.* (1995). Ethanol and isopentanol increase CYP3A and CYP2E in primary cultures of human hepatocytes. *Arch Biochem Biophys*, 322: 516–520. doi:10.1006/abbi.1995.1495 PMID:7574728

Kristiansen E, Clemmensen S, Meyer O (1990). Chronic ethanol intake and reduction of lung tumours from urethane in strain A mice. *Food Chem Toxicol*, 28: 35–38. doi:10.1016/0278-6915(90)90133-8 PMID:2138114

Kruysse A, Feron VJ, Til HP (1975). Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters. *Arch Environ Health*, 30: 449–452. PMID:1164047

Kucheria K, Taneja N, Mohan D (1986). Chromosomal aberrations and sister chromatid exchanges in chronic male alcoholics. *Indian J Med Res*, 83: 417–421. PMID:3721549

Kurtz AJ & Lloyd RS (2003). 1,N2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. *J Biol Chem*, 278: 5970–5976. doi:10.1074/jbc.M212012200 PMID:12502710

Kurys G, Ambroziak W, Pietruszko R (1989). Human aldehyde dehydrogenase. Purification and characterization of a third isozyme with low Km for gamma-aminobutyraldehyde. *J Biol Chem*, 264: 4715–4721. PMID:2925663

Kuykendall JR & Bogdanffy MS (1992). Reaction kinetics of DNA–histone crosslinking by vinyl acetate and acetaldehyde. *Carcinogenesis*, 13: 2095–2100. doi:10.1093/carcin/13.11.2095 PMID:1423881

Kwo PY, Ramchandani VA, O'Connor S *et al.* (1998). Gender differences in alcohol metabolism: relationship to liver volume and effect of adjusting for body mass. *Gastroenterology*, 115: 1552–1557. doi:10.1016/S0016-5085(98)70035-6 PMID:9834284

Ladero JM, Agúndez JAG, Rodríguez-Lescure A *et al.* (1996). RsaI polymorphism at the cytochrome P4502E1 locus and risk of hepatocellular carcinoma. *Gut*, 39: 330–333. doi:10.1136/gut.39.2.330 PMID:8977352

Lagadic-Gossmann D, Lerche C, Rissel M *et al.* (2000). The induction of the human hepatic CYP2E1 gene by interleukin 4 is transcriptional and regulated by protein kinase C. *Cell Biol Toxicol*, 16: 221–233. doi:10.1023/A:1007625925095 PMID:11101004

Lähdetie J (1988). Effects of vinyl acetate and acetaldehyde on sperm morphology and meiotic micronuclei in mice. *Mutat Res*, 202: 171–178. PMID:3185588

Laheij RJF, Verlaan M, Van Oijen MGH *et al.* (2004). Gastrointestinal symptoms and ethanol metabolism in alcoholics. *Dig Dis Sci*, 49: 1007–1011. doi:10.1023/B:DDAS.0000034563.02099.78 PMID:15309892

Lam C-W, Casanova M, Heck HD (1986). Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure. *Fundam Appl Toxicol*, 6: 541–550. doi:10.1016/0272-0590(86)90228-9 PMID:3699337

Lamarche F, Gonthier B, Signorini N *et al.* (2003). Acute exposure of cultured neurones to ethanol results in reversible DNA single-strand breaks; whereas chronic exposure causes loss of cell viability. *Alcohol Alcohol*, 38: 550–558. PMID:14633642

Lamarche F, Gonthier B, Signorini N *et al.* (2004). Impact of ethanol and acetaldehyde on DNA and cell viability of cultured neurones. *Cell Biol Toxicol*, 20: 361–374. doi:10.1007/s10565-004-0087-9 PMID:15868480

Lambert B, Chen Y, He S-M, Sten M (1985). DNA cross-links in human leucocytes treated with vinyl acetate and acetaldehyde in vitro. *Mutat Res*, 146: 301–303. PMID:4058447

Landi S, Gemignani F, Moreno V *et al.*Bellvitge Colorectal Cancer Study Group. (2005). A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. *Pharmacogenet Genomics*, 15: 535–546. doi:10.1097/01.fpc.0000165904.48994.3d PMID:16006997

Laposata EA & Lange LG (1986). Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse. *Science*, 231: 497–499. doi:10.1126/science.3941913 PMID:3941913

Laposata M, Hasaba A, Best CA *et al.* (2002). Fatty acid ethyl esters: recent observations. *Prostaglandins Leukot Essent Fatty Acids*, 67: 193–196. doi:10.1054/plef.2002.0418 PMID:12324241

Larkby C & Day N (1997). The effects of prenatal alcohol exposure. *Alcohol Health Res World*, 21: 192–198. PMID:15706768

Larsson SC, Giovannucci E, Wolk A (2006). Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology*, 131: 1271–1283. doi:10.1053/j.gastro.2006.08.010 PMID:17030196

Lau CF, Vogel R, Obe G, Spielmann H (1991). Embryologic and cytogenetic effects of ethanol on preimplantation mouse embryos in vitro. *Reprod Toxicol*, 5: 405–410. doi:10.1016/0890-6238(91)90003-X PMID:1806149

Le Marchand L, Donlon T, Hankin JH *et al.* (2002). B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Causes Control*, 13: 239–248. doi:10.1023/A:1015057614870 PMID:12020105

Le Marchand L, Haiman CA, Wilkens LR *et al.* (2004). MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*, 13: 2071–2077. PMID:15598763

Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE (2005). The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*, 14: 1198–1203. doi:10.1158/1055-9965.EPI-04-0840 PMID:15894672

Lee CH, Lee JM, Wu DC *et al.* (2005). Independent and combined effects of alcohol intake, tobacco smoking and betel quid chewing on the risk of esophageal cancer in Taiwan. *Int J Cancer*, 113: 475–482. doi:10.1002/ijc.20619 PMID:15455377

Lee H-S, Yoon J-H, Kamimura S *et al.* (1997). Lack of association of cytochrome P450 2E1 genetic polymorphisms with the risk of human hepatocellular carcinoma. *Int J Cancer*, 71: 737–740. doi:10.1002/(SICI)1097-0215(19970529)71:5<737::AID-IJC8>3.0.CO;2-S PMID:9180139

Lee J-M, Lee Y-C, Yang S-Y *et al.* (2001). Genetic polymorphisms of XRCC1 and risk of the esophageal cancer. *Int J Cancer*, 95: 240–246. doi:10.1002/1097-0215(20010720)95:4<240::AID-IJC1041>3.0.CO;2-1 PMID:11400117

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*, 30: 1132–1142. doi:10.1111/j.1530-0277.2006.00139.x PMID:16792560

Leo MA & Lieber CS (1999). Alcohol, vitamin A, and beta-carotene: adverse interactions, including hepatotoxicity and carcinogenicity. *Am J Clin Nutr*, 69: 1071–1085. PMID:10357725

Levine AJ, Siegmund KD, Ervin CM *et al.* (2000). The methylenetetrahydrofolate reductase 677C–>T polymorphism and distal colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev*, 9: 657–663. PMID:10919734

Levitt MD, Furne J, DeMaster E (1997b). First-pass metabolism of ethanol is negligible in rat gastric mucosa. *Alcohol Clin Exp Res*, 21: 293–297. PMID:9113266

Levitt MD & Levitt DG (1998). Use of a two-compartment model to assess the pharmacokinetics of human ethanol metabolism. *Alcohol Clin Exp Res*, 22: 1680–1688. PMID:9835281

Levitt MD & Levitt DG (2000). Appropriate use and misuse of blood concentration measurements to quantitate first-pass metabolism. *J Lab Clin Med*, 136: 275–280. doi:10.1067/mlc.2000.109100 PMID:11039847

Levitt MD, Levitt DG, Furne J, DeMaster EG (1994). Can the liver account for first-pass metabolism of ethanol in the rat? *Am J Physiol*, 267: G452–G457. PMID:7943243

Levitt MD, Li R, DeMaster EG *et al.* (1997a). Use of measurements of ethanol absorption from stomach and intestine to assess human ethanol metabolism. *Am J Physiol*, 273: G951–G957. PMID:9357840

Lewis SJ, Harbord RM, Harris R, Smith GD (2006). Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. *J Natl Cancer Inst*, 98: 1607–1622. doi:10.1093/jnci/djj440 PMID:17105984

Lewis SJ & Smith GD (2005). Alcohol, ALDH2, and esophageal cancer: a meta-analysis which illustrates the potentials and limitations of a Mendelian randomization approach. *Cancer Epidemiol Biomarkers Prev*, 14: 1967–1971. doi:10.1158/1055-9965.EPI-05-0196 PMID:16103445

Li D, Ahmed M, Li Y *et al.* (2005b). 5,10-Methylenetetrahydrofolate reductase polymorphisms and the risk of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*, 14: 1470–1476. doi:10.1158/1055-9965.EPI-04-0894 PMID:15941958

Li D, Dandara C, Parker MI (2005a). Association of cytochrome P450 2E1 genetic polymorphisms with squamous cell carcinoma of the oesophagus. *Clin Chem Lab Med*, 43: 370–375. doi:10.1515/CCLM.2005.067 PMID:15899651

Li J, French BA, Fu P *et al.* (2003). Mechanism of the alcohol cyclic pattern: role of catecholamines. *Am J Physiol Gastrointest Liver Physiol*, 285: G442–G448. PMID:12702493

Li J, Nguyen V, French BA *et al.* (2000). Mechanism of the alcohol cyclic pattern: role of the hypothalamic-pituitary-thyroid axis. *Am J Physiol Gastrointest Liver Physiol*, 279: G118–G125. PMID:10898753

Liangpunsakul S, Kolwankar D, Pinto A *et al.* (2005). Activity of CYP2E1 and CYP3A enzymes in adults with moderate alcohol consumption: a comparison with nonalcoholics. *Hepatology*, 41: 1144–1150. doi:10.1002/hep.20673 PMID:15841467

Lieber CS (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)–A review. *Alcohol Clin Exp Res*, 23: 991–1007. doi:10.1111/j.1530-0277.1999.tb04217.x PMID:10397283

Lieber CS (2002). S-Adenosyl-L-methionine and alcoholic liver disease in animal models: implications for early intervention in human beings. *Alcohol*, 27: 173–177. doi:10.1016/S0741-8329(02)00230-6 PMID:12163146

Lieber CS (2004a). The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. *Drug Metab Rev*, 36: 511–529. doi:10.1081/DMR-200033441 PMID:15554233

Lieber CS (2004b). Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol*, 34: 9–19. doi:10.1016/j.alcohol.2004.07.008 PMID:15670660

Lieber CS, Baraona E, Leo MA, Garro A (1987). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper No. 15/2. Metabolism and metabolic effects of ethanol, including interaction with drugs, carcinogens and nutrition. *Mutat Res*, 186: 201–233. PMID:3313028

Lieber CS, Robins SJ, Li J *et al.* (1994). Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology*, 106: 152–159. PMID:8276177

Lilla C, Koehler T, Kropp S *et al.* (2005). Alcohol dehydrogenase 1B (ADH1B) genotype, alcohol consumption and breast cancer risk by age 50 years in a German case-control study. *Br J Cancer*, 92: 2039–2041. doi:10.1038/sj.bjc.6602608 PMID:15886702

Lilly LJ (1975). Investigations in bitro and in vivo, of the effects of disulfiram (Antabuse) on human lymphocyte chromosomes. *Toxicology*, 4: 331–340. doi:10.1016/0300-483X(75)90055-4 PMID:1154430

Lim RT Jr, Gentry RT, Ito D *et al.* (1993). First-pass metabolism of ethanol is predominantly gastric. *Alcohol Clin Exp Res*, 17: 1337–1344. doi:10.1111/j.1530-0277.1993.tb05250.x PMID:8116851

Lin D-X, Tang Y-M, Peng Q *et al.* (1998). Susceptibility to esophageal cancer and genetic polymorphisms in glutathione S-transferases T1, P1, and M1 and cytochrome P450 2E1. *Cancer Epidemiol Biomarkers Prev*, 7: 1013–1018. PMID:9829710

Lin SW, Chen JC, Hsu LC *et al.* (1996). Human gamma-aminobutyraldehyde dehydrogenase (ALDH9): cDNA sequence, genomic organization, polymorphism, chromosomal localization, and tissue expression. *Genomics*, 34: 376–380. doi:10.1006/geno.1996.0300 PMID:8786138

Lin YC, Ho IC, Lee TC (1989). Ethanol and acetaldehyde potentiate the clastogenicity of ultraviolet light, methyl methanesulfonate, mitomycin C and bleomycin in Chinese hamster ovary cells. *Mutat Res*, 216: 93–99. PMID:2467201

Little RE & Wendt JK (1991). The effects of maternal drinking in the reproductive period: an epidemiologic review. *J Subst Abuse*, 3: 187–204. doi:10.1016/S0899-3289(05)80036-7 PMID:1821281

Liu SY & Gonzalez FJ (1995). Role of the liver-enriched transcription factor HNF-1 alpha in expression of the CYP2E1 gene. *DNA Cell Biol*, 14: 285–293. doi:10.1089/dna.1995.14.285 PMID:7710685

Liu X, Lao Y, Yang IY *et al.* (2006). Replication-coupled repair of crotonaldehyde/acetaldehyde-induced guanine–guanine interstrand cross-links and their mutagenicity. *Biochemistry*, 45: 12898–12905. doi:10.1021/bi060792v PMID:17042508

Lodovici M, Casalini C, Cariaggi R *et al.* (2000). Levels of 8-hydroxydeoxyguanosine as a marker of DNA damage in human leukocytes. *Free Radic Biol Med*, 28: 13–17. doi:10.1016/S0891-5849(99)00194-X PMID:10656286

Loizou GD & Cocker J (2001). The effects of alcohol and diallyl sulphide on CYP2E1 activity in humans: a phenotyping study using chlorzoxazone. *Hum Exp Toxicol*, 20: 321–327. doi:10.1191/096032701680350587 PMID:11530830

Louis CA, Wood SG, Kostrubsky V *et al.* (1994). Synergistic increases in rat hepatic cytochrome P450s by ethanol and isopentanol. *J Pharmacol Exp Ther*, 269: 838–845. PMID:8182553

Lu X-M, Zhang Y-M, Lin R-Y *et al.* (2005). Relationship between genetic polymorphisms of metabolizing enzymes CYP2E1, GSTM1 and Kazakh's esophageal squamous cell cancer in Xinjiang, China. *World J Gastroenterol*, 11: 3651–3654. PMID:15968714

Lucas D, Ménez C, Floch F *et al.* (1996). Cytochromes P4502E1 and P4501A1 genotypes and susceptibility to cirrhosis or upper aerodigestive tract cancer in alcoholic caucasians. *Alcohol Clin Exp Res*, 20: 1033–1037. doi:10.1111/j.1530-0277.1996.tb01943.x PMID:8892524

Lucas D, Ménez C, Girre C *et al.* (1995). Decrease in cytochrome P4502E1 as assessed by the rate of chlorzoxazone hydroxylation in alcoholics during the withdrawal phase. *Alcohol Clin Exp Res*, 19: 362–366. doi:10.1111/j.1530-0277.1995.tb01516.x PMID:7625570

Lucock M (2000). Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab*, 71: 121–138. doi:10.1006/mgme.2000.3027 PMID:11001804

Luedemann C, Bord E, Qin G *et al.* (2005). Ethanol modulation of TNF-alpha biosynthesis and signaling in endothelial cells: synergistic augmentation of TNF-alpha mediated endothelial cell dysfunctions by chronic ethanol. *Alcohol Clin Exp Res*, 29: 930–938. doi:10.1097/01.ALC.0000171037.90100.6B PMID:15976518

Lumeng L, Bosron WF, Li TK (1979). Quantitative correlation of ethanol elimination rates in vivo with liver alcohol dehydrogenase activities in fed, fasted and food-restricted rats. *Biochem Pharmacol*, 28: 1547–1551. doi:10.1016/0006-2952(79)90471-4 PMID:475866

Ma J, Stampfer MJ, Christensen B *et al.* (1999). A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*, 8: 825–829. PMID:10498402

Ma J, Stampfer MJ, Giovannucci E *et al.* (1997). Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res*, 57: 1098–1102. PMID:9067278

MacGregor RR (1986). Alcohol and immune defense. *JAMA*, 256: 1474–1479. doi:10.1001/jama.256.11.1474 PMID:3747066

Maffei F, Fimognari C, Castelli E *et al.* (2000). Increased cytogenetic damage detected by FISH analysis on micronuclei in peripheral lymphocytes from alcoholics. *Mutagenesis*, 15: 517–523. doi:10.1093/mutage/15.6.517 PMID:11077004

Maffei F, Forti GC, Castelli E *et al.* (2002). Biomarkers to assess the genetic damage induced by alcohol abuse in human lymphocytes. *Mutat Res*, 514: 49–58. PMID:11815244

Mahabir S, Baer DJ, Johnson LL *et al.* (2004). The effects of moderate alcohol supplementation on estrone sulfate and DHEAS in postmenopausal women in a controlled feeding study. *Nutr J*, 3: 11 doi:10.1186/1475-2891-3-11 PMID:15353002

Maier H, Born IA, Veith S *et al.* (1986). The effect of chronic ethanol consumption on salivary gland morphology and function in the rat. *Alcohol Clin Exp Res*, 10: 425–427. doi:10.1111/j.1530-0277.1986.tb05117.x PMID:3530020

Majer BJ, Mersch-Sundermann V, Darroudi F *et al.* (2004). Genotoxic effects of dietary and lifestyle related carcinogens in human derived hepatoma (HepG2, Hep3B) cells. *Mutat Res*, 551: 153–166. PMID:15225590

Majumdar AP, Vesenka GD, Dubick MA *et al.* (1986). Morphological and biochemical changes of the pancreas in rats treated with acetaldehyde. *Am J Physiol*, 250: G598–G606. PMID:2422952

Manari AP, Preedy VR, Peters TJ (2003). Nutritional intake of hazardous drinkers and dependent alcoholics in the UK. *Addict Biol*, 8: 201–210. doi:10.1080/1355621031000117437 PMID:12850779

Mandayam S, Jamal MM, Morgan TR (2004). Epidemiology of alcoholic liver disease. *Semin Liver Dis*, 24: 217–232. doi:10.1055/s-2004-832936 PMID:15349801

Mandola MV, Stoehlmacher J, Zhang W *et al.* (2004). A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. *Pharmacogenetics*, 14: 319–327. doi:10.1097/00008571-200405000-00007 PMID:15115918

Mao H, Reddy GR, Marnett LJ, Stone MP (1999). Solution structure of an oligodeoxynucleotide containing the malondialdehyde deoxyguanosine adduct N2-(3-oxo-1-propenyl)-dG (ring-opened M1G) positioned in a (CpG)3 frameshift hotspot of the Salmonella typhimurium hisD3052 gene. *Biochemistry*, 38: 13491–13501. doi:10.1021/bi9910124 PMID:10521256

Marmot MG (1984). Alcohol and coronary heart disease. *Int J Epidemiol*, 13: 160–167. doi:10.1093/ije/13.2.160 PMID:6376385

Marmot MG (2001). Alcohol and coronary heart disease. *Int J Epidemiol*, 30: 724–729. doi:10.1093/ije/30.4.724 PMID:11511592

Marnett LJ, Hurd HK, Hollstein MC *et al.* (1985). Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. *Mutat Res*, 148: 25–34. PMID:3881660

Martin NG, Perl J, Oakeshott JG *et al.* (1985). A twin study of ethanol metabolism. *Behav Genet*, 15: 93–109. doi:10.1007/BF01065891 PMID:3838073

Martinez F, Abril ER, Earnest DL, Watson RR (1992). Ethanol and cytokine secretion. *Alcohol*, 9: 455–458. doi:10.1016/0741-8329(92)90080-T PMID:1472299

Marugame T, Tsuji E, Kiyohara C *et al.* (2003). Relation of plasma folate and methylenetetrahydrofolate reductase C677T polymorphism to colorectal adenomas. *Int J Epidemiol*, 32: 64–66. doi:10.1093/ije/dyg004 PMID:12690011

Mason JB & Choi SW (2005). Effects of alcohol on folate metabolism: implications for carcinogenesis. *Alcohol*, 35: 235–241. doi:10.1016/j.alcohol.2005.03.012 PMID:16054985

Matsuda T, Kawanishi M, Yagi T *et al.* (1998). Specific tandem GG to TT base substitutions induced by acetaldehyde are due to intra-strand crosslinks between adjacent guanine bases. *Nucleic Acids Res*, 26: 1769–1774. doi:10.1093/nar/26.7.1769 PMID:9512551

Matsuda T, Terashima I, Matsumoto Y *et al.* (1999). Effective utilization of N2-ethyl-2′-deoxyguanosine triphosphate during DNA synthesis catalyzed by mammalian replicative DNA polymerases. *Biochemistry*, 38: 929–935. doi:10.1021/bi982134j PMID:9893988

Matsuda T, Yabushita H, Kanaly RA *et al.* (2006). Increased DNA damage in ALDH2-deficient alcoholics. *Chem Res Toxicol*, 19: 1374–1378. doi:10.1021/tx060113h PMID:17040107

Matsuda Y, Baraona E, Salaspuro M, Lieber CS (1979). Effects of ethanol on liver microtubules and Golgi apparatus. Possible role in altered hepatic secretion of plasma proteins. *Lab Invest*, 41: 455–463. PMID:502475

Matsuda Y, Takada A, Sato H *et al.* (1985). Comparison between ballooned hepatocytes occurring in human alcoholic and nonalcoholic liver diseases. *Alcohol Clin Exp Res*, 9: 366–370. doi:10.1111/j.1530-0277.1985.tb05561.x PMID:3901809

Matsumoto H & Fukui Y (2002). Pharmacokinetics of ethanol: a review of the methodology. *Addict Biol*, 7: 5–14. doi:10.1080/135562101200100553 PMID:11900618

Matsumoto H, Matsubayashi K, Fukui Y (1994). Estimation of ethanol first-pass effect in the perfused rat liver. *Alcohol Alcohol Suppl*, 29: 9–13. PMID:9063826

Matsumoto H, Matsubayashi K, Fukui Y (1996). Evidence that cytochrome P-4502E1 contributes to ethanol elimination at low doses: effects of diallyl sulfide and 4-methyl pyrazole on ethanol elimination in the perfused rat liver. *Alcohol Clin Exp Res*, 20: Suppl 112A–16A. doi:10.1111/j.1530-0277.1996.tb01719.x PMID:8659679

Matsumoto H, Minowa Y, Nishitani Y, Fukui Y (1999). An allometric model for predicting blood ethanol elimination in mammals. *Biochem Pharmacol*, 57: 219–223. doi:10.1016/S0006-2952(98)00292-5 PMID:9890571

Matsuo K, Hamajima N, Hirai T *et al.* (2002). Aldehyde dehydrogenase 2 (ALDH2) genotype affects rectal cancer susceptibility due to alcohol consumption. *J Epidemiol*, 12: 70–76. PMID:12033531

Matsuo K, Hamajima N, Shinoda M *et al.* (2001). Gene-environment interaction between an aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. *Carcinogenesis*, 22: 913–916. doi:10.1093/carcin/22.6.913 PMID:11375898

Matsuo K, Ito H, Wakai K *et al.* (2005). One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. *Carcinogenesis*, 26: 2164–2171. doi:10.1093/carcin/bgi196 PMID:16051637

Matsuo K, Wakai K, Hirose K *et al.* (2006a). A gene-gene interaction between ALDH2 Glu487Lys and ADH2 His47Arg polymorphisms regarding the risk of colorectal cancer in Japan. *Carcinogenesis*, 27: 1018–1023. doi:10.1093/carcin/bgi282 PMID:16332725

Matsuo K, Wakai K, Hirose K *et al.* (2006b). Alcohol dehydrogenase 2 His47Arg polymorphism influences drinking habit independently of aldehyde dehydrogenase 2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects. *Cancer Epidemiol Biomarkers Prev*, 15: 1009–1013. doi:10.1158/1055-9965.EPI-05-0911 PMID:16702384

Matsushima Y (1987). Chromosomal aberrations in the lymphocytes of alcoholics and former alcoholics. *Neuropsychobiology*, 17: 24–29. doi:10.1159/000118336 PMID:3627389

Matthias C, Bockmühl U, Jahnke V *et al.* (1998). Polymorphism in cytochrome P450 CYP2D6, CYP1A1, CYP2E1 and glutathione S-transferase, GSTM1, GSTM3, GSTT1 and susceptibility to tobacco-related cancers: studies in upper aerodigestive tract cancers. *Pharmacogenetics*, 8: 91–100. PMID:10022746

Mattson SN, Schoenfeld AM, Riley EP (2001). Teratogenic effects of alcohol on brain and behavior. *Alcohol Res Health*, 25: 185–191. PMID:11810956

McCarver DG, Byun R, Hines RN *et al.* (1998). A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicol Appl Pharmacol*, 152: 276–281. doi:10.1006/taap.1998.8532 PMID:9772223

McCoy GD, Hecht SS, Furuya K (1986). The effect of chronic ethanol consumption on the tumorigenicity of N-nitrosopyrrolidine in male Syrian golden hamsters. *Cancer Lett*, 33: 151–159. doi:10.1016/0304-3835(86)90019-4 PMID:3791185

McCoy GD, Hecht SS, Katayama S, Wynder EL (1981). Differential effect of chronic ethanol consumption on the carcinogenicity of N-nitrosopyrrolidine and N'-nitrosonornicotine in male Syrian golden hamsters. *Cancer Res*, 41: 2849–2854. PMID:7248945

McGehee RE Jr, Ronis MJJ, Cowherd RM *et al.* (1994). Characterization of cytochrome P450 2E1 induction in a rat hepatoma FGC-4 cell model by ethanol. *Biochem Pharmacol*, 48: 1823–1833. doi:10.1016/0006-2952(94)90469-3 PMID:7980652

McKim SE, Uesugi T, Raleigh JA *et al.* (2003). Chronic intragastric alcohol exposure causes hypoxia and oxidative stress in the rat pancreas. *Arch Biochem Biophys*, 417: 34–43. doi:10.1016/S0003-9861(03)00349-7 PMID:12921777

McKinnon RA & McManus ME (1996). Localization of cytochromes P450 in human tissues: implications for chemical toxicity. *Pathology*, 28: 148–155. doi:10.1080/00313029600169783 PMID:8743822

Mello NK, Mendelson JH, Teoh SK (1989). Neuroendocrine consequences of alcohol abuse in women. *Ann N Y Acad Sci*, 562: 1 Prenatal Abus211–240. doi:10.1111/j.1749-6632.1989.tb21020.x PMID:2662859

Mendelson JH, Lukas SE, Mello NK *et al.* (1988). Acute alcohol effects on plasma estradiol levels in women. *Psychopharmacology (Berl)*, 94: 464–467. doi:10.1007/BF00212838 PMID:3131791

Mendelson JH & Mello NK (1988). Chronic alcohol effects on anterior pituitary and ovarian hormones in healthy women. *J Pharmacol Exp Ther*, 245: 407–412. PMID:3367299

Meskar A, Plee-Gautier E, Amet Y *et al.* (2001). Alcohol-xenobiotic interactions. Role of cytochrome P450 2E1 *Pathol Biol (Paris)*, 49: 696–702. PMID:11762131

Mezey E, Oesterling JE, Potter JJ (1988). Influence of male hormones on rates of ethanol elimination in man. *Hepatology*, 8: 742–744. doi:10.1002/hep.1840080406 PMID:3391502

Mezey E & Potter JJ (1979). Rat liver alcohol dehydrogenase activity: effects of growth hormone and hypophysectomy. *Endocrinology*, 104: 1667–1673. doi:10.1210/endo-104-6-1667 PMID:221187

Migliore L, Cocchi L, Scarpato R (1996). Detection of the centromere in micronuclei by fluorescence in situ hybridization: its application to the human lymphocyte micronucleus assay after treatment with four suspected aneugens. *Mutagenesis*, 11: 285–290. doi:10.1093/mutage/11.3.285 PMID:8671750

Mitelman F & Wadstein J (1978). Chromosome aberrations in chronic alcoholics. *Lancet*, 1: 216 doi:10.1016/S0140-6736(78)90659-1 PMID:74645

Mizoi Y, Ijiri I, Tatsuno Y *et al.* (1979). Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. *Pharmacol Biochem Behav*, 10: 303–311. doi:10.1016/0091-3057(79)90105-9 PMID:450943

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*, 29: 707–710. PMID:7695788

Moon K-H, Hood BL, Kim B-J *et al.* (2006). Inactivation of oxidized and S-nitrosylated mitochondrial proteins in alcoholic fatty liver of rats. *Hepatology*, 44: 1218–1230. doi:10.1002/hep.21372 PMID:17058263

Moore S, Montane-Jaime K, Shafe S *et al.* (2007). Association of ALDH1 promoter polymorphisms with alcohol-related phenotypes in Trinidad and Tobago. *J Stud Alcohol Drugs*, 68: 192–196. PMID:17286337

Morgan TR, Mandayam S, Jamal MM (2004). Alcohol and hepatocellular carcinoma. *Gastroenterology*, 127: Suppl 1S87–S96. doi:10.1053/j.gastro.2004.09.020 PMID:15508108

Morimoto K & Takeshita T (1996). Low Km aldehyde dehydrogenase (ALDH2) polymorphism, alcohol-drinking behavior, and chromosome alterations in peripheral lymphocytes. *Environ Health Perspect*, 104: Suppl 3563–567. doi:10.2307/3432824 PMID:8781384

Morita M, Oyama T, Kagawa N *et al.* (2005). Expression of aldehyde dehydrogenase 2 in the normal esophageal epithelium and alcohol consumption in patients with esophageal cancer. *Front Biosci*, 10: 2319–2324. doi:10.2741/1700 PMID:15970497

Morita S, Yano M, Shiozaki H *et al.* (1997). CYP1A1, CYP2E1 and GSTM1 polymorphisms are not associated with susceptibility to squamous-cell carcinoma of the esophagus. *Int J Cancer*, 71: 192–195. doi:10.1002/(SICI)1097-0215(19970410)71:2<192::AID-IJC11>3.0.CO;2-K PMID:9139841

Mortelmans K, Haworth S, Lawlor T *et al.* (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen*, 8: Suppl 71–119. PMID:3516675

Mufti SI (1998). Alcohol-stimulated promotion of tumors in the gastrointestinal tract. *Cancer Detect Prev*, 22: 195–203. doi:10.1046/j.1525-1500.1998.00023.x PMID:9618040

Mufti SI, Darban HR, Watson RR (1989). Alcohol, cancer, and immunomodulation. *Crit Rev Oncol Hematol*, 9: 243–261. doi:10.1016/S1040-8428(89)80003-4 PMID:2686698

Mulligan CJ, Robin RW, Osier MV *et al.* (2003). Allelic variation at alcohol metabolism genes (ADH1B, ADH1C, ALDH2) and alcohol dependence in an American Indian population. *Hum Genet*, 113: 325–336. doi:10.1007/s00439-003-0971-z PMID:12884000

Munaka M, Kohshi K, Kawamoto T *et al.* (2003). Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol*, 129: 355–360. doi:10.1007/s00432-003-0439-5 PMID:12759747

Murata M, Tagawa M, Watanabe S *et al.* (1999). Genotype difference of aldehyde dehydrogenase 2 gene in alcohol drinkers influences the incidence of Japanese colorectal cancer patients. *Jpn J Cancer Res*, 90: 711–719. PMID:10470282

Muto M, Nakane M, Hitomi Y *et al.* (2002). Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. *Carcinogenesis*, 23: 1759–1765. doi:10.1093/carcin/23.10.1759 PMID:12376487

Muto M, Takahashi M, Ohtsu A *et al.* (2005). Risk of multiple squamous cell carcinomas both in the esophagus and the head and neck region. *Carcinogenesis*, 26: 1008–1012. doi:10.1093/carcin/bgi035 PMID:15718256

Nakajima M, Takeuchi T, Takeshita T, Morimoto K (1996). 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: effects of individual alcohol sensitivity. *Environ Health Perspect*, 104: 1336–1338. doi:10.2307/3432971 PMID:9118876

Nakajima T, Okuyama S, Yonekura I, Sato A (1985). Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene. *Chem Biol Interact*, 55: 23–38. doi:10.1016/S0009-2797(85)80118-6 PMID:4064192

Nakashima H, Yamamoto M, Goto K *et al.* (1989). Isolation and characterization of the rat catalase-encoding gene. *Gene*, 79: 279–288. doi:10.1016/0378-1119(89)90210-2 PMID:2792765

National Toxicology Program (2004) *Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/ethanol* (Technical Report 510; NIH Publication No. 04-4444), Research Triangle Park, NC.

Navasumrit P, Margison GP, O'Connor PJ (2001a). Ethanol modulates rat hepatic DNA repair functions. *Alcohol Alcohol*, 36: 369–376. PMID:11524300

Navasumrit P, Ward TH, Dodd NJ, O'Connor PJ (2000). Ethanol-induced free radicals and hepatic DNA strand breaks are prevented in vivo by antioxidants: effects of acute and chronic ethanol exposure. *Carcinogenesis*, 21: 93–99. doi:10.1093/carcin/21.1.93 PMID:10607739

Navasumrit P, Ward TH, O'Connor PJ *et al.* (2001b). Ethanol enhances the formation of endogenously and exogenously derived adducts in rat hepatic DNA. *Mutat Res*, 479: 81–94. PMID:11470483

Neumark YD, Friedlander Y, Durst R *et al.* (2004). Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. *Alcohol Clin Exp Res*, 28: 10–14. doi:10.1097/01.ALC.0000108667.79219.4D PMID:14745297

Neumark YD, Friedlander Y, Thomasson HR, Li TK (1998). Association of the ADH2*2 allele with reduced ethanol consumption in Jewish men in Israel: a pilot study. *J Stud Alcohol*, 59: 133–139. PMID:9500299

Nishimoto IN, Pinheiro NA, Rogatto SR *et al.* (2004). Alcohol dehydrogenase 3 genotype as a risk factor for upper aerodigestive tract cancers. *Arch Otolaryngol Head Neck Surg*, 130: 78–82. doi:10.1001/archotol.130.1.78 PMID:14732773

Nishimura M, Yaguti H, Yoshitsugu H *et al.* (2003). Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi*, 123: 369–375. doi:10.1248/yakushi.123.369 PMID:12772594

Nomura T, Noma H, Shibahara T *et al.* (2000). Aldehyde dehydrogenase 2 and glutathione S-transferase M 1 polymorphisms in relation to the risk for oral cancer in Japanese drinkers. *Oral Oncol*, 36: 42–46. doi:10.1016/S1368-8375(99)00048-2 PMID:10889918

Norppa H, Tursi F, Pfäffli P *et al.* (1985). Chromosome damage induced by vinyl acetate through in vitro formation of acetaldehyde in human lymphocytes and Chinese hamster ovary cells. *Cancer Res*, 45: 4816–4821. PMID:4027971

Norton ID, Apte MV, Lux O *et al.* (1998). Chronic ethanol administration causes oxidative stress in the rat pancreas. *J Lab Clin Med*, 131: 442–446. doi:10.1016/S0022-2143(98)90145-7 PMID:9605109

Novak RF & Woodcroft KJ (2000). The alcohol-inducible form of cytochrome P450 (CYP 2E1): role in toxicology and regulation of expression. *Arch Pharm Res*, 23: 267–282. doi:10.1007/BF02975435 PMID:10976571

Novoradovsky A, Tsai S-JL, Goldfarb L *et al.* (1995a). Mitochondrial aldehyde dehydrogenase polymorphism in Asian and American Indian populations: detection of new ALDH2 alleles. *Alcohol Clin Exp Res*, 19: 1105–1110. doi:10.1111/j.1530-0277.1995.tb01587.x PMID:8561277

Novoradovsky AG, Sandoval C, Guderian RH *et al.* (1995b). Detection of aldehyde dehydrogenase deficiency in Chachi Indians, Ecuador. *Alcohol*, 12: 159–161. doi:10.1016/0741-8329(94)00085-9 PMID:7772268

Nuutinen H, Lindros KO, Salaspuro M (1983). Determinants of blood acetaldehyde level during ethanol oxidation in chronic alcoholics. *Alcohol Clin Exp Res*, 7: 163–168. doi:10.1111/j.1530-0277.1983.tb05432.x PMID:6346918

Nuutinen HU, Salaspuro MP, Valle M, Lindros KO (1984). Blood acetaldehyde concentration gradient between hepatic and antecubital venous blood in ethanol-intoxicated alcoholics and controls. *Eur J Clin Invest*, 14: 306–311. doi:10.1111/j.1365-2362.1984.tb01186.x PMID:6434326

O'Connor S, Morzorati S, Christian J, Li T-K (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. *Alcohol Clin Exp Res*, 22: 202–210. doi:10.1111/j.1530-0277.1998.tb03639.x PMID:9514308

O'Hare ED, Kan E, Yoshii J *et al.* (2005). Mapping cerebellar vermal morphology and cognitive correlates in prenatal alcohol exposure. *Neuroreport*, 16: 1285–1290. doi:10.1097/01.wnr.0000176515.11723.a2 PMID:16056126

O'Neill GT & Kaufman MH (1987). Cytogenetic analysis of first cleavage fertilized mouse eggs following in vivo exposure to ethanol shortly before and at the time of conception. *Development*, 100: 441–448. PMID:3652980

O'Shea KS & Kaufman MH (1979). The teratogenic effect of acetaldehyde: implications for the study of the fetal alcohol syndrome. *J Anat*, 128: 65–76. PMID:422485

O'Shea KS & Kaufman MH (1981). Effect of acetaldehyde on the neuroepithelium of early mouse embryos. *J Anat*, 132: 107–118. PMID:7275785

Obe G & Anderson D (1987). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper No. 15/1. Genetic effects of ethanol. *Mutat Res*, 186: 177–200. PMID:3313027

Obe G & Beek B (1979). Mutagenic activity of aldehydes. *Drug Alcohol Depend*, 4: 91–94. doi:10.1016/0376-8716(79)90044-9 PMID:574448

Obe G, Göbel D, Engeln H *et al.* (1980). Chromosomal aberrations in peripheral lymphocytes of alcoholics. *Mutat Res*, 73: 377–386. PMID:7464846

Obe G, Jonas R, Schmidt S (1986). Metabolism of ethanol in vitro produces a compound which induces sister-chromatid exchanges in human peripheral lymphocytes in vitro: acetaldehyde not ethanol is mutagenic. *Mutat Res*, 174: 47–51. doi:10.1016/0165-7992(86)90075-8 PMID:2939344

Obe G, Natarajan AT, Meyers M, Hertog AD (1979). Induction of chromosomal aberrations in peripheral lymphocytes of human blood in vitro, and of SCEs in bone-

marrow cells of mice in vivo by ethanol and its metabolite acetaldehyde. *Mutat Res*, 68: 291–294. doi:10.1016/0165-1218(79)90160-5 PMID:514307

Obe G & Ristow H (1977). Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. *Mutat Res*, 56: 211–213.

Obe G, Ristow H, Herha J (1978). Mutagenic activity of alcohol in man. In: *Mutations: Their Origin, Nature and Potential Relevance to Genetic Risk in Man*, Boppard, Harald Boldt, pp. 151–161.

Oda Y, Aryal P, Terashita T *et al.* (2001). Metabolic activation of heterocyclic amines and other procarcinogens in Salmonella typhimurium umu tester strains expressing human cytochrome P4501A1, 1A2, 1B1, 2C9, 2D6, 2E1, and 3A4 and human NADPH-P450 reductase and bacterial O-acetyltransferase. *Mutat Res*, 492: 81–90. PMID:11377247

Oesch-Bartlomowicz B, Padma PR, Becker R *et al.* (1998). Differential modulation of CYP2E1 activity by cAMP-dependent protein kinase upon Ser129 replacement. *Exp Cell Res*, 242: 294–302. doi:10.1006/excr.1998.4120 PMID:9665827

Ohhira M, Fujimoto Y, Matsumoto A *et al.* (1996). Hepatocellular carcinoma associated with alcoholic liver disease: a clinicopathological study and genetic polymorphism of aldehyde dehydrogenase 2. *Alcohol Clin Exp Res*, 20: Suppl378A–382A. PMID:8986242

Olshan AF, Weissler MC, Watson MA, Bell DA (2001). Risk of head and neck cancer and the alcohol dehydrogenase 3 genotype. *Carcinogenesis*, 22: 57–61. doi:10.1093/carcin/22.1.57 PMID:11159741

Oneta CM, Simanowski UA, Martinez M *et al.* (1998). First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. *Gut*, 43: 612–619. doi:10.1136/gut.43.5.612 PMID:9824340

Orellana M, Rodrigo R, Valdés E (1998). Peroxisomal and microsomal fatty acid oxidation in liver of rats after chronic ethanol consumption. *Gen Pharmacol*, 31: 817–820. PMID:9809485

Oshita M, Sato N, Yoshihara H *et al.* (1992). Ethanol-induced vasoconstriction causes focal hepatocellular injury in the isolated perfused rat liver. *Hepatology*, 16: 1007–1013. doi:10.1002/hep.1840160425 PMID:1398480

Osier MV, Pakstis AJ, Goldman D *et al.* (2002). A proline–threonine substitution in codon 351 of ADH1C is common in Native Americans. *Alcohol Clin Exp Res*, 26: 1759–1763. PMID:12500098

Otani T, Iwasaki M, Hanaoka T *et al.* (2005). Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. *Nutr Cancer*, 53: 42–50. doi:10.1207/s15327914nc5301_5 PMID:16351505

Oyama T, Isse T, Kagawa N *et al.* (2005). Tissue-distribution of aldehyde dehydrogenase 2 and effects of the ALDH2 gene-disruption on the expression of enzymes involved in alcohol metabolism. *Front Biosci*, 10: 951–960. doi:10.2741/1589 PMID:15569633

Ozawa S, Ohta K, Miyajima A *et al.* (2000). Metabolic activation of o-phenylphenol to a major cytotoxic metabolite, phenylhydroquinone: role of human CYP1A2 and rat CYP2C11/CYP2E1. *Xenobiotica*, 30: 1005–1017. doi:10.1080/00498250050200159 PMID:11315102

Pandya GA & Moriya M (1996). 1,N6-ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. *Biochemistry*, 35: 11487–11492. doi:10.1021/bi960170h PMID:8784204

Panés J, Soler X, Parés A *et al.* (1989). Influence of liver disease on hepatic alcohol and aldehyde dehydrogenases. *Gastroenterology*, 97: 708–714. PMID:2753331

Parés X, Cederlund E, Moreno A *et al.* (1994). Mammalian class IV alcohol dehydrogenase (stomach alcohol dehydrogenase): structure, origin, and correlation with enzymology. *Proc Natl Acad Sci U S A*, 91: 1893–1897. doi:10.1073/pnas.91.5.1893 PMID:8127901

Park K-K, Liem A, Stewart BC, Miller JA (1993). Vinyl carbamate epoxide, a major strong electrophilic, mutagenic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate (urethane). *Carcinogenesis*, 14: 441–450. doi:10.1093/carcin/14.3.441 PMID:8453720

Parlesak A, Billinger MH-U, Bode C, Bode JC (2002). Gastric alcohol dehydrogenase activity in man: influence of gender, age, alcohol consumption and smoking in a caucasian population. *Alcohol Alcohol*, 37: 388–393. PMID:12107043

Pastino GM & Conolly RB (2000). Application of a physiologically based pharmacokinetic model to estimate the bioavailability of ethanol in male rats: distinction between gastric and hepatic pathways of metabolic clearance. *Toxicol Sci*, 55: 256–265. doi:10.1093/toxsci/55.2.256 PMID:10828256

Pastino GM, Sultatos LG, Flynn EJ (1996). Development and application of a physiologically based pharmacokinetic model for ethanol in the mouse. *Alcohol Alcohol*, 31: 365–374. PMID:8879283

Pavia CS, La Mothe M, Kavanagh M (2004). Influence of alcohol on antimicrobial immunity. *Biomed Pharmacother*, 58: 84–89. doi:10.1016/j.biopha.2003.12.005 PMID:14992788

Pedrosa MC, Russell RM, Saltzman JR *et al.* (1996). Gastric emptying and first-pass metabolism of ethanol in elderly subjects with and without atrophic gastritis. *Scand J Gastroenterol*, 31: 671–677. doi:10.3109/00365529609009148 PMID:8819216

Peters ES, McClean MD, Liu M *et al.* (2005). The ADH1C polymorphism modifies the risk of squamous cell carcinoma of the head and neck associated with alcohol and tobacco use. *Cancer Epidemiol Biomarkers Prev*, 14: 476–482. doi:10.1158/1055-9965.EPI-04-0431 PMID:15734975

Pfeiffer A, Högl B, Kaess H (1992). Effect of ethanol and commonly ingested alcoholic beverages on gastric emptying and gastrointestinal transit. *Clin Investig*, 70: 487–491. doi:10.1007/BF00210229 PMID:1392416

Phillips BJ & Jenkinson P (2001). Is ethanol genotoxic? A review of the published data. *Mutagenesis*, 16: 91–101. doi:10.1093/mutage/16.2.91 PMID:11230549

Pikkarainen PH & Lieber CS (1980). Concentration dependency of ethanol elimination rates in baboons: effect of chronic alcohol consumption. *Alcohol Clin Exp Res*, 4: 40–43. doi:10.1111/j.1530-0277.1980.tb04789.x PMID:6766681

Piña Calva A & Madrigal-Bujaidar E (1993). SCE frequencies induced by ethanol, tequila and brandy in mouse bone marrow cells in vivo. *Toxicol Lett*, 66: 1–5. doi:10.1016/0378-4274(93)90072-6 PMID:8427015

Pinaire J, Chou W-Y, Stewart M *et al.* (1999). Activity of the human aldehyde dehydrogenase 2 promoter is influenced by the balance between activation by hepatocyte nuclear factor 4 and repression by perosixome proliferator activated receptor delta, chicken ovalbumin upstream promoter-transcription factor, and apolipoprotein regulatory protein-1. *Adv Exp Med Biol*, 463: 115–121. PMID:10352676

Pluskota-Karwatka D, Pawłowicz AJ, Kronberg L (2006). Formation of malonaldehyde-acetaldehyde conjugate adducts in calf thymus DNA. *Chem Res Toxicol*, 19: 921–926. doi:10.1021/tx060027h PMID:16841960

Polygenis D, Wharton S, Malmberg C *et al.* (1998). Moderate alcohol consumption during pregnancy and the incidence of fetal malformations: a meta-analysis. *Neurotoxicol Teratol*, 20: 61–67. doi:10.1016/S0892-0362(97)00073-1 PMID:9511170

Ponnappa BC, Hoek JB, Jubinski L, Rubin E (1988). Effect of chronic ethanol ingestion on pancreatic protein synthesis. *Biochim Biophys Acta*, 966: 390–402. PMID:3416016

Pool-Zobel BL, Dornacher I, Lambertz R *et al.* (2004). Genetic damage and repair in human rectal cells for biomonitoring: sex differences, effects of alcohol exposure, and susceptibilities in comparison to peripheral blood lymphocytes. *Mutat Res*, 551: 127–134. PMID:15225587

Popov VB, Vaisman BL, Puchkov VF, Ignat'eva TV (1981). Embryotoxic effect of ethanol and its biotransformation products in cultures of postimplantation rat embryos *Biull Eksp Biol Med*, 92: 725–728. PMID:7326425

Porasuphatana S, Weaver J, Rosen GM (2006). Inducible nitric oxide synthase catalyzes ethanol oxidation to alpha-hydroxyethyl radical and acetaldehyde. *Toxicology*, 223: 167–174. doi:10.1016/j.tox.2006.02.022 PMID:16713055

Pöschl G & Seitz HK (2004). Alcohol and cancer. *Alcohol Alcohol*, 39: 155–165. PMID:15082451

Potter JJ, Rennie-Tankersley L, Mezey E (2003). Endotoxin enhances liver alcohol dehydrogenase by action through upstream stimulatory factor but not by nuclear factor-kappa B. *J Biol Chem*, 278: 4353–4357. doi:10.1074/jbc.M210097200 PMID:12454009

Potter JJ, Yang VW, Mezey E (1993). Regulation of the rat class I alcohol dehydrogenase gene by growth hormone. *Biochem Biophys Res Commun*, 191: 1040–1045. doi:10.1006/bbrc.1993.1322 PMID:8466483

Powell H, Kitteringham NR, Pirmohamed M *et al.* (1998). Expression of cytochrome P4502E1 in human liver: assessment by mRNA, genotype and pheno-

type. *Pharmacogenetics*, 8: 411–421. doi:10.1097/00008571-199810000-00006 PMID:9825833

Prasanna CV, Namagiri T, Ramakrishnan S (1986). Effect of acetaldehyde on thyroid function. *Indian J Exp Biol*, 24: 77–78. PMID:3733169

Prasanna CV & Ramakrishnan S (1987). Effect of acetaldehyde on hepatic and blood lipids. *Indian J Med Res*, 85: 206–211. PMID:3596689

Prasanna CV & Ramakrishnan S (1984). Effect of acetaldehyde on hepatic glucose metabolism. *Indian J Biochem Biophys*, 21: 62–64. PMID:6490059

Pronko P, Bardina L, Satanovskaya V *et al.* (2002). Effect of chronic alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. *Alcohol Alcohol*, 37: 229–235. PMID:12003909

Puddey IB, Rakic V, Dimmitt SB, Beilin LJ (1999). Influence of pattern of drinking on cardiovascular disease and cardiovascular risk factors–A review. *Addiction*, 94: 649–663. doi:10.1046/j.1360-0443.1999.9456493.x PMID:10563030

Pylkkänen L & Salonen I (1987). Concomitant mutagenicity of ethanol and x-ray irradiation in the mouse male germ cells. *Alcohol*, 4: 401–404. doi:10.1016/0741-8329(87)90074-7 PMID:3675862

Qiu LO, Linder MW, Antonino-Green DM, Valdes R Jr (2004). Suppression of cytochrome P450 2E1 promoter activity by interferon-gamma and loss of response due to the -71G>T nucleotide polymorphism of the CYP2E1*7B allele. *J Pharmacol Exp Ther*, 308: 284–288. doi:10.1124/jpet.103.057208 PMID:14566010

Quertemont E (2004). Genetic polymorphism in ethanol metabolism: acetaldehyde contribution to alcohol abuse and alcoholism. *Mol Psychiatry*, 9: 570–581. doi:10.1038/sj.mp.4001497 PMID:15164086

Rachamin G, MacDonald JA, Wahid S *et al.* (1980). Modulation of alcohol dehydrogenase and ethanol metabolism by sex hormones in the spontaneously hypertensive rat. Effect of chronic ethanol administration. *Biochem J*, 186: 483–490. PMID:6990919

Raha S & Robinson BH (2000). Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci*, 25: 502–508. doi:10.1016/S0968-0004(00)01674-1 PMID:11050436

Rajah TT & Ahuja YR (1996). In vivo genotoxicity of alcohol consumption and lead exposure in printing press workers. *Alcohol*, 13: 65–68. doi:10.1016/0741-8329(95)02014-4 PMID:8837937

Rajendram R & Preedy VR (2005). Effect of alcohol consumption on the gut. *Dig Dis*, 23: 214–221. doi:10.1159/000090168 PMID:16508285

Ramchandani VA, Kwo PY, Li T-K (2001). Effect of food and food composition on alcohol elimination rates in healthy men and women. *J Clin Pharmacol*, 41: 1345–1350. doi:10.1177/00912700122012814 PMID:11762562

Ramirez A & Saldanha PH (2002). Micronucleus investigation of alcoholic patients with oral carcinomas. *Genet Mol Res*, 1: 246–260. PMID:14963832

Rao UN, Aravindakshan M, Chauhan PS (1994). Studies on the effect of ethanol on dominant lethal mutations in Swiss, C57BL6 and CBA mice. *Mutat Res*, 311: 69–76. PMID:7526176

Raucy JL, Curley G, Carpenter SP (1995). Use of lymphocytes for assessing ethanol-mediated alterations in the expression of hepatic cytochrome P4502E1. *Alcohol Clin Exp Res*, 19: 1369–1375. doi:10.1111/j.1530-0277.1995.tb00994.x PMID:8749797

Raucy JL, Kraner JC, Lasker JM (1993). Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit Rev Toxicol*, 23: 1–20. doi:10.3109/10408449309104072 PMID:8471158

Raucy JL, Lasker J, Ozaki K, Zoleta V (2004). Regulation of CYP2E1 by ethanol and palmitic acid and CYP4A11 by clofibrate in primary cultures of human hepatocytes. *Toxicol Sci*, 79: 233–241. doi:10.1093/toxsci/kfh126 PMID:15056802

Raucy JL, Schultz ED, Kearins MC *et al.* (1999). CYP2E1 expression in human lymphocytes from various ethnic populations. *Alcohol Clin Exp Res*, 23: 1868–1874. PMID:10630604

Raucy JL, Schultz ED, Wester MR *et al.* (1997). Human lymphocyte cytochrome P450 2E1, a putative marker for alcohol-mediated changes in hepatic chlorzoxazone activity. *Drug Metab Dispos*, 25: 1429–1435. PMID:9394034

Regan TJ, Ettinger PO, Haider B *et al.* (1977). The role of ethanol in cardiac disease. *Annu Rev Med*, 28: 393–409. doi:10.1146/annurev.me.28.020177.002141 PMID:324369

Reichman ME, Judd JT, Longcope C *et al.* (1993). Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. *J Natl Cancer Inst*, 85: 722–727. doi:10.1093/jnci/85.9.722 PMID:8478958

Reynolds K, Lewis B, Nolen JD *et al.* (2003). Alcohol consumption and risk of stroke: a meta-analysis. *JAMA*, 289: 579–588. doi:10.1001/jama.289.5.579 PMID:12578491

Rimm EB, Klatsky A, Grobbee D, Stampfer MJ (1996). Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. *BMJ*, 312: 731–736. PMID:8605457

Rinaldi S, Peeters PH, Bezemer ID *et al.* (2006). Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition. *Cancer Causes Control*, 17: 1033–1043. doi:10.1007/s10552-006-0041-7 PMID:16933054

Risch A, Ramroth H, Raedts V *et al.* (2003). Laryngeal cancer risk in Caucasians is associated with alcohol and tobacco consumption but not modified by genetic polymorphisms in class I alcohol dehydrogenases ADH1B and ADH1C, and glutathione-S-transferases GSTM1 and GSTT1. *Pharmacogenetics*, 13: 225–230. doi:10.1097/00008571-200304000-00007 PMID:12668919

Ristow H & Obe G (1978). Acetaldehyde induces cross-links in DNA and causes sister-chromatid exchanges in human cells. *Mutat Res*, 58: 115–119. doi:10.1016/0165-1218(78)90103-9 PMID:714076

Ristow H, Seyfarth A, Lochmann ER (1995). Chromosomal damages by ethanol and acetaldehyde in Saccharomyces cerevisiae as studied by pulsed field gel electrophoresis. *Mutat Res*, 326: 165–170. PMID:7529880

Robbins WA, Vine MF, Truong KY, Everson RB (1997). Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. *Environ Mol Mutagen*, 30: 175–183. doi:10.1002/(SICI)1098-2280(1997)30:2<175::AID-EM10>3.0.CO;2-A PMID:9329642

Roberts BJ, Shoaf SE, Jeong K-S, Song B-J (1994). Induction of CYP2E1 in liver, kidney, brain and intestine during chronic ethanol administration and withdrawal: evidence that CYP2E1 possesses a rapid phase half-life of 6 hours or less. *Biochem Biophys Res Commun*, 205: 1064–1071. doi:10.1006/bbrc.1994.2774 PMID:7802633

Roberts BJ, Shoaf SE, Song BJ (1995b). Rapid changes in cytochrome P4502E1 (CYP2E1) activity and other P450 isozymes following ethanol withdrawal in rats. *Biochem Pharmacol*, 49: 1665–1673. doi:10.1016/0006-2952(95)00098-K PMID:7786308

Roberts BJ, Song B-J, Soh Y *et al.* (1995a). Ethanol induces CYP2E1 by protein stabilization. Role of ubiquitin conjugation in the rapid degradation of CYP2E1. *J Biol Chem*, 270: 29632–29635. PMID:8530344

Roine R (2000). Interaction of prandial state and beverage concentration on alcohol absorption. *Alcohol Clin Exp Res*, 24: 411–412. doi:10.1111/j.1530-0277.2000.tb02000.x PMID:10798569

Roine R, Gentry RT, Hernández-Munõz R *et al.* (1990). Aspirin increases blood alcohol concentrations in humans after ingestion of ethanol. *JAMA*, 264: 2406–2408. doi:10.1001/jama.264.18.2406 PMID:2231997

Roine RP, Gentry RT, Lim RT Jr *et al.* (1991). Effect of concentration of ingested ethanol on blood alcohol levels. *Alcohol Clin Exp Res*, 15: 734–738. doi:10.1111/j.1530-0277.1991.tb00589.x PMID:1928652

Roine RP, Gentry RT, Lim RT Jr *et al.* (1993). Comparison of blood alcohol concentrations after beer and whiskey. *Alcohol Clin Exp Res*, 17: 709–711. doi:10.1111/j.1530-0277.1993.tb00824.x PMID:8333604

Ronen GM & Andrews WL (1991). Holoprosencephaly as a possible embryonic alcohol effect. *Am J Med Genet*, 40: 151–154. doi:10.1002/ajmg.1320400206 PMID:1897567

Ronis MJ, Huang J, Ingelman-Sundberg M, Badger TM (1991). Effect of nutrition and alcohol on the microsomal monooxygenase system of rat kidney. *FASEB J*, 5: A936

Ronis MJJ, Huang J, Crouch J *et al.* (1993). Cytochrome P450 CYP 2E1 induction during chronic alcohol exposure occurs by a two-step mechanism associated with blood alcohol concentrations in rats. *J Pharmacol Exp Ther*, 264: 944–950. PMID:8437134

Rosenkranz HS (1977). Mutagenicity of halogenated alkanes and their derivatives. *Environ Health Perspect*, 21: 79–84. doi:10.2307/3428504 PMID:348460

Rowlands JC, Wang H, Hakkak R *et al.* (2000). Chronic intragastric infusion of ethanol-containing diets induces CYP3A9 while decreasing CYP3A2 in male rats. *J Pharmacol Exp Ther*, 295: 747–752. PMID:11046114

Saffroy R, Pham P, Chiappini F *et al.* (2004). The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. *Carcinogenesis*, 25: 1443–1448. doi:10.1093/carcin/bgh147 PMID:15033905

Sakai Y, Yamaji T, Tabata S *et al.* (2006). Relation of alcohol use and smoking to glucose tolerance status in Japanese men. *Diabetes Res Clin Pract*, 73: 83–88. doi:10.1016/j.diabres.2005.12.010 PMID:16494963

Sakamoto T, Hara M, Higaki Y *et al.* (2006). Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. *Int J Cancer*, 118: 1501–1507. doi:10.1002/ijc.21505 PMID:16187278

Sako M, Inagaki S, Esaka Y, Deyashiki Y (2003). Histones accelerate the cyclic 1,N2-propanoguanine adduct-formation of DNA by the primary metabolite of alcohol and carcinogenic crotonaldehyde. *Bioorg Med Chem Lett*, 13: 3497–3498. doi:10.1016/S0960-894X(03)00800-X PMID:14505656

Saladino AJ, Willey JC, Lechner JF *et al.* (1985). Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. *Cancer Res*, 45: 2522–2526. PMID:3986791

Salaspuro M (1996). Bacteriocolonic pathway for ethanol oxidation: characteristics and implications. *Ann Med*, 28: 195–200. doi:10.3109/07853899609033120 PMID:8811162

Salaspuro MP (2003). Alcohol consumption and cancer of the gastrointestinal tract. *Best Pract Res Clin Gastroenterol*, 17: 679–694. doi:10.1016/S1521-6918(03)00035-0 PMID:12828962

Saldiva PHN, do Rio Caldeira MP, Massad E *et al.* (1985). Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. *J Appl Toxicol*, 5: 288–292. doi:10.1002/jat.2550050505 PMID:4056306

Salmela KS, Kessova IG, Tsyrlov IB, Lieber CS (1998). Respective roles of human cytochrome P-4502E1, 1A2, and 3A4 in the hepatic microsomal ethanol oxidizing system. *Alcohol Clin Exp Res*, 22: 2125–2132. doi:10.1111/j.1530-0277.1998.tb05926.x PMID:9884161

Sapag A, Tampier L, Valle-Prieto A *et al.* (2003). Mutations in mitochondrial aldehyde dehydrogenase (ALDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. *Pharmacogenetics*, 13: 509–515. doi:10.1097/00008571-200308000-00009 PMID:12893989

Sarkola T, Adlercreutz H, Heinonen S *et al.* (2001). The role of the liver in the acute effect of alcohol on androgens in women. *J Clin Endocrinol Metab*, 86: 1981–1985. doi:10.1210/jc.86.5.1981 PMID:11344195

Sarkola T & Eriksson CJ (2003). Testosterone increases in men after a low dose of alcohol. *Alcohol Clin Exp Res*, 27: 682–685. PMID:12711931

Sarkola T, Fukunaga T, Mäkisalo H, Peter Eriksson CJ (2000). Acute effect of alcohol on androgens in premenopausal women. *Alcohol Alcohol*, 35: 84–90. PMID:10684783

Sarkola T, Mäkisalo H, Fukunaga T, Eriksson CJ (1999). Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol Clin Exp Res*, 23: 976–982. PMID:10397281

Savitz DA, Schwingl PJ, Keels MA (1991). Influence of paternal age, smoking, and alcohol consumption on congenital anomalies. *Teratology*, 44: 429–440. doi:10.1002/tera.1420440409 PMID:1962288

Schmähl D, Krüger FW, Habs M, Diehl B (1976). Influence of disulfiram on the organotropy of the carcinogenic effect of dimethylnitrosamine and diethylnitrosamine in rats. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol*, 85: 271–276. doi:10.1007/BF00284085 PMID:131427

Schoedel KA, Sellers EM, Tyndale RF (2001). Induction of CYP2B1/2 and nicotine metabolism by ethanol in rat liver but not rat brain. *Biochem Pharmacol*, 62: 1025–1036. doi:10.1016/S0006-2952(01)00744-4 PMID:11597571

Schwartz SM, Doody DR, Fitzgibbons ED *et al.* (2001). Oral squamous cell cancer risk in relation to alcohol consumption and alcohol dehydrogenase-3 genotypes. *Cancer Epidemiol Biomarkers Prev*, 10: 1137–1144. PMID:11700261

Seitz HK, Egerer G, Oneta C *et al.* (1996). Alcohol dehydrogenase in the human colon and rectum. *Digestion*, 57: 105–108. doi:10.1159/000201322 PMID:8785998

Seitz HK, Egerer G, Simanowski UA *et al.* (1993). Human gastric alcohol dehydrogenase activity: effect of age, sex, and alcoholism. *Gut*, 34: 1433–1437. doi:10.1136/gut.34.10.1433 PMID:8244116

Seitz HK, Simanowski UA, Garzon FT *et al.* (1990). Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. *Gastroenterology*, 98: 406–413. PMID:2295396

Seitz HK & Stickel F (2006). Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem*, 387: 349–360. doi:10.1515/BC.2006.047 PMID:16606331

Seshadri R, Baker E, Sutherland GR (1982). Sister-chromatid exchange (SCE) analysis in mothers exposed to DNA-damaging agents and their newborn infants. *Mutat Res*, 97: 139–146. PMID:6210844

Sharp L & Little J (2004). Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol*, 159: 423–443. doi:10.1093/aje/kwh066 PMID:14977639

Shaw S, Jayatilleke E, Herbert V, Colman N (1989). Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem J*, 257: 277–280. PMID:2537625

Shea SH, Wall TL, Carr LG, Li T-K (2001). ADH2 and alcohol-related phenotypes in Ashkenazic Jewish American college students. *Behav Genet*, 31: 231–239. doi:10.1023/A:1010261713092 PMID:11545539

Shen H, Sturgis EM, Khan SG *et al.* (2001). An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: a case–control study. *Cancer Res*, 61: 3321–3325. PMID:11309287

Sherman DIN, Ward RJ, Warren-Perry M *et al.* (1993a). Association of restriction fragment length polymorphism in alcohol dehydrogenase 2 gene with alcohol induced liver damage. *BMJ*, 307: 1388–1390. doi:10.1136/bmj.307.6916.1388 PMID:7903883

Sherman D, Davé V, Hsu LC *et al.* (1993b). Diverse polymorphism within a short coding region of the human aldehyde dehydrogenase-5 (ALDH5) gene. *Hum Genet*, 92: 477–480. doi:10.1007/BF00216454 PMID:8244338

Shibata A, Fukuda K, Nishiyori A *et al.* (1998). A case–control study on male hepatocellular carcinoma based on hospital and community controls. *J Epidemiol*, 8: 1–5. PMID:9575688

Shimizu M, Lasker JM, Tsutsumi M, Lieber CS (1990). Immunohistochemical localization of ethanol-inducible P450IIE1 in the rat alimentary tract. *Gastroenterology*, 99: 1044–1053. PMID:2203661

Shrubsole MJ, Gao Y-T, Cai Q *et al.* (2004). MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev*, 13: 190–196. doi:10.1158/1055-9965.EPI-03-0273 PMID:14973091

Siegmund S, Haas S, Schneider A, Singer MV (2003). Animal models in gastrointestinal alcohol research-a short appraisal of the different models and their results. *Best Pract Res Clin Gastroenterol*, 17: 519–542. doi:10.1016/S1521-6918(03)00033-7 PMID:12828953

Siegmund SV & Brenner DA (2005). Molecular pathogenesis of alcohol-induced hepatic fibrosis. *Alcohol Clin Exp Res*, 29: Suppl102S–109S. doi:10.1097/01.alc.0000189275.97419.58 PMID:16344593

Sierksma A, Sarkola T, Eriksson CJ *et al.* (2004). Effect of moderate alcohol consumption on plasma dehydroepiandrosterone sulfate, testosterone, and estradiol levels in middle-aged men and postmenopausal women: a diet-controlled intervention study. *Alcohol Clin Exp Res*, 28: 780–785. doi:10.1097/01.ALC.0000125356.70824.81 PMID:15166654

Simanowski UA, Homann N, Knühl M *et al.* (2001). Increased rectal cell proliferation following alcohol abuse. *Gut*, 49: 418–422. doi:10.1136/gut.49.3.418 PMID:11511565

Simanowski UA, Seitz HK, Baier B *et al.* (1986). Chronic ethanol consumption selectively stimulates rectal cell proliferation in the rat. *Gut*, 27: 278–282. doi:10.1136/gut.27.3.278 PMID:3699547

Simanowski UA, Stickel F, Maier H *et al.* (1995). Effect of alcohol on gastrointestinal cell regeneration as a possible mechanism in alcohol-associated carcinogenesis. *Alcohol*, 12: 111–115. doi:10.1016/0741-8329(94)00091-3 PMID:7772260

Sinclair J, Jeffery E, Wrighton S *et al.* (1998). Alcohol-mediated increases in acetaminophen hepatotoxicity: role of CYP2E and CYP3A. *Biochem Pharmacol*, 55: 1557–1565. PMID:9633991

Sinclair J, Lambrecht L, Smith EL (1990). Hepatic alcohol dehydrogenase activity in chick hepatocytes towards the major alcohols present in commercial alcoholic beverages: comparison with activities in rat and human liver. *Comp Biochem Physiol B*, 96: 677–682. PMID:2225771

Sinclair JF, McCaffrey J, Sinclair PR *et al.* (1991). Ethanol increases cytochromes P450IIE, IIB1/2, and IIIA in cultured rat hepatocytes. *Arch Biochem Biophys*, 284: 360–365. doi:10.1016/0003-9861(91)90308-6 PMID:1989519

Singh NP & Khan A (1995). Acetaldehyde: genotoxicity and cytotoxicity in human lymphocytes. *Mutat Res*, 337: 9–17. PMID:7596360

Singh NP, Lai H, Khan A (1995). Ethanol-induced single-strand DNA breaks in rat brain cells. *Mutat Res*, 345: 191–196. doi:10.1016/0165-1218(95)90054-3 PMID:8552140

Singletary KW & Gapstur SM (2001). Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *JAMA*, 286: 2143–2151. doi:10.1001/jama.286.17.2143 PMID:11694156

Sipi P, Järventaus H, Norppa H (1992). Sister-chromatid exchanges induced by vinyl esters and respective carboxylic acids in cultured human lymphocytes. *Mutat Res*, 279: 75–82. doi:10.1016/0165-1218(92)90248-X PMID:1375341

Slattery ML, Potter JD, Samowitz W *et al.* (1999). Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*, 8: 513–518. PMID:10385141

Smith IE, Coles CD, Lancaster J *et al.* (1986). The effect of volume and duration of prenatal ethanol exposure on neonatal physical and behavioral development. *Neurobehav Toxicol Teratol*, 8: 375–381. PMID:3762847

Smith T, DeMaster EG, Furne JK *et al.* (1992). First-pass gastric mucosal metabolism of ethanol is negligible in the rat. *J Clin Invest*, 89: 1801–1806. doi:10.1172/JCI115784 PMID:1601990

Sohn OS, Fiala ES, Requeijo SP *et al.* (2001). Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer Res*, 61: 8435–8440. PMID:11731424

Sowell ER, Thompson PM, Peterson BS *et al.* (2002). Mapping cortical gray matter asymmetry patterns in adolescents with heavy prenatal alcohol exposure. *Neuroimage*, 17: 1807–1819. doi:10.1006/nimg.2002.1328 PMID:12498754

Spence JP, Liang T, Eriksson CJP *et al.* (2003). Evaluation of aldehyde dehydrogenase 1 promoter polymorphisms identified in human populations. *Alcohol Clin Exp Res*, 27: 1389–1394. doi:10.1097/01.ALC.0000087086.50089.59 PMID:14506398

Sreenathan RN, Padmanabhan R, Singh S (1984). Structural changes of placenta following maternal administration of acetaldehyde in rat. *Z Mikrosk Anat Forsch*, 98: 597–604. PMID:6524011

Sreerama L & Sladek NE (1997). Cellular levels of class 1 and class 3 aldehyde dehydrogenases and certain other drug-metabolizing enzymes in human breast malignancies. *Clin Cancer Res*, 3: 1901–1914. PMID:9815579

Stein S, Lao Y, Yang IY *et al.* (2006). Genotoxicity of acetaldehyde- and crotonaldehyde-induced 1,N2-propanodeoxyguanosine DNA adducts in human cells. *Mutat Res*, 608: 1–7. PMID:16797223

Steinmetz CG, Xie P, Weiner H, Hurley TD (1997). Structure of mitochondrial aldehyde dehydrogenase: the genetic component of ethanol aversion. *Structure*, 5: 701–711. doi:10.1016/S0969-2126(97)00224-4 PMID:9195888

Stevens RG, Davis S, Mirick DK *et al.* (2000). Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. *Epidemiology*, 11: 660–665. doi:10.1097/00001648-200011000-00008 PMID:11055626

Stewart MJ, Dipple KM, Stewart TR, Crabb DW (1996b). The role of nuclear factor NF-Y/CP1 in the transcriptional regulation of the human aldehyde dehydrogenase 2-encoding gene. *Gene*, 173: 155–161. doi:10.1016/0378-1119(96)00068-6 PMID:8964492

Stewart MJ, Malek K, Crabb DW (1996a). Distribution of messenger RNAs for aldehyde dehydrogenase 1, aldehyde dehydrogenase 2, and aldehyde dehydrogenase 5 in human tissues. *J Investig Med*, 44: 42–46. PMID:8689400

Stich HF & Rosin MP (1983). Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. *Int J Cancer*, 31: 305–308. doi:10.1002/ijc.2910310309 PMID:6826255

Stickel F & Österreicher CH (2006). The role of genetic polymorphisms in alcoholic liver disease. *Alcohol Alcohol*, 41: 209–224. PMID:16492723

Stickel F, Schuppan D, Hahn EG, Seitz HK (2002). Cocarcinogenic effects of alcohol in hepatocarcinogenesis. *Gut*, 51: 132–139. doi:10.1136/gut.51.1.132 PMID:12077107

Stolzenberg-Solomon RZ, Qiao YL, Abnet CC *et al.* (2003). Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev*, 12: 1222–1226. PMID:14652285

Streissguth AP, Aase JM, Clarren SK *et al.* (1991a). Fetal alcohol syndrome in adolescents and adults. *JAMA*, 265: 1961–1967. doi:10.1001/jama.265.15.1961 PMID:2008025

Streissguth AP, Randels SP, Smith DF (1991b). A test-retest study of intelligence in patients with fetal alcohol syndrome: implications for care. *J Am Acad Child Adolesc Psychiatry*, 30: 584–587. doi:10.1097/00004583-199107000-00009 PMID:1823538

Strömberg P, Svensson S, Hedberg JJ *et al.* (2002). Identification and characterisation of two allelic forms of human alcohol dehydrogenase 2. *Cell Mol Life Sci*, 59: 552–559. doi:10.1007/s00018-002-8447-1 PMID:11964133

Sturgis EM, Castillo EJ, Li L *et al.* (1999). Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. *Carcinogenesis*, 20: 2125–2129. doi:10.1093/carcin/20.11.2125 PMID:10545415

Sturgis EM, Dahlstrom KR, Guan Y *et al.* (2001). Alcohol dehydrogenase 3 genotype is not associated with risk of squamous cell carcinoma of the oral cavity and pharynx. *Cancer Epidemiol Biomarkers Prev*, 10: 273–275. PMID:11303599

Sturgis EM, Zheng R, Li L *et al.* (2000). XPD/ERCC2 polymorphisms and risk of head and neck cancer: a case–control analysis. *Carcinogenesis*, 21: 2219–2223. doi:10.1093/carcin/21.12.2219 PMID:11133811

Sugimura T, Kumimoto H, Tohnai I *et al.* (2006). Gene–environment interaction involved in oral carcinogenesis: molecular epidemiological study for metabolic and DNA repair gene polymorphisms. *J Oral Pathol Med*, 35: 11–18. doi:10.1111/j.1600-0714.2005.00364.x PMID:16393248

Svensson S, Strömberg P, Höög J-O (1999). A novel subtype of class II alcohol dehydrogenase in rodents. Unique Pro(47) and Ser(182) modulates hydride transfer in the mouse enzyme. *J Biol Chem*, 274: 29712–29719. doi:10.1074/jbc.274.42.29712 PMID:10514444

Swann PF, Coe AM, Mace R (1984). Ethanol and dimethylnitrosamine and diethylnitrosamine metabolism and disposition in the rat. Possible relevance to the influence of ethanol on human cancer incidence. *Carcinogenesis*, 5: 1337–1343. doi:10.1093/carcin/5.10.1337 PMID:6435899

Swann PF, Graves RJ, Mace R (1987). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper No. 15/6. Effect of ethanol on nitrosamine metabolism and distribution. Implications for the role of nitrosamines in human cancer and for the influence of alcohol consumption on cancer incidence. *Mutat Res*, 186: 261–267. PMID:3313032

Sydow K, Daiber A, Oelze M *et al.* (2004). Central role of mitochondrial aldehyde dehydrogenase and reactive oxygen species in nitroglycerin tolerance and cross-tolerance. *J Clin Invest*, 113: 482–489. PMID:14755345

Szabo G (1997). Alcohol's contribution to compromised immunity. *Alcohol Health Res World*, 21: 30–41. PMID:15706761

Szabo G (1999). Consequences of alcohol consumption on host defence. *Alcohol Alcohol*, 34: 830–841. PMID:10659718

Takahashi T, Lasker JM, Rosman AS, Lieber CS (1993). Induction of cytochrome P-4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology*, 17: 236–245. PMID:8428720

Takeshita T, Mao X-Q, Morimoto K (1996). The contribution of polymorphism in the alcohol dehydrogenase β subunit to alcohol sensitivity in a Japanese population. *Hum Genet*, 97: 409–413. doi:10.1007/BF02267057 PMID:8834233

Takeshita T, Morimoto K, Yamaguchi N *et al.* (2000b). Relationships between cigarette smoking, alcohol drinking, the ALDH2 genotype and adenomatous types of colorectal polyps in male self-defense force officials. *J Epidemiol*, 10: 366–371. PMID:11210104

Takeshita T, Yang X, Inoue Y *et al.* (2000a). Relationship between alcohol drinking, ADH2 and ALDH2 genotypes, and risk for hepatocellular carcinoma in Japanese. *Cancer Lett*, 149: 69–76. doi:10.1016/S0304-3835(99)00343-2 PMID:10737710

Takezaki T, Gao C-M, Wu J-Z *et al.* (2002). hOGG1 Ser(326)Cys polymorphism and modification by environmental factors of stomach cancer risk in Chinese. *Int J Cancer*, 99: 624–627. doi:10.1002/ijc.10400 PMID:11992556

Tan W, Song N, Wang G-Q *et al.* (2000). Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. *Cancer Epidemiol Biomarkers Prev*, 9: 551–556. PMID:10868687

Tanabe H, Ohhira M, Ohtsubo T *et al.* (1999). Genetic polymorphism of aldehyde dehydrogenase 2 in patients with upper aerodigestive tract cancer. *Alcohol Clin Exp Res*, 23: Suppl17S–20S. doi:10.1111/j.1530-0277.1999.tb04527.x PMID:10235272

Tanaka F, Shiratori Y, Yokosuka O *et al.* (1996). High incidence of ADH2*1/ALDH2*1 genes among Japanese alcohol dependents and patients with alcoholic liver disease. *Hepatology*, 23: 234–239. doi:10.1002/hep.510230206 PMID:8591846

Tarter RE, Hegedus AM, Goldstein G *et al.* (1984). Adolescent sons of alcoholics: neuropsychological and personality characteristics. *Alcohol Clin Exp Res*, 8: 216–222. doi:10.1111/j.1530-0277.1984.tb05842.x PMID:6375434

Tavares DC, Cecchi AO, Jordão AA Jr *et al.* (2001). Cytogenetic study of chronic ethanol consumption in rats. *Teratog Carcinog Mutagen*, 21: 361–368. doi:10.1002/tcm.1024 PMID:11746250

Teo AK, Oh HK, Ali RB, Li BF (2001). The modified human DNA repair enzyme O^6-methylguanine–DNA methyltransferase is a negative regulator of estrogen receptor-mediated transcription upon alkylation DNA damage. *Mol Cell Biol*, 21: 7105–7114. doi:10.1128/MCB.21.20.7105-7114.2001 PMID:11564893

Terelius Y, Norsten-Höög C, Cronholm T, Ingelman-Sundberg M (1991). Acetaldehyde as a substrate for ethanol-inducible cytochrome P450 (CYP2E1). *Biochem Biophys Res Commun*, 179: 689–694. doi:10.1016/0006-291X(91)91427-E PMID:1822117

Terry MB, Gammon MD, Zhang FF *et al.* (2006). ADH3 genotype, alcohol intake and breast cancer risk. *Carcinogenesis*, 27: 840–847. doi:10.1093/carcin/bgi285 PMID:16344274

Testa M, Quigley BM, Eiden RD (2003). The effects of prenatal alcohol exposure on infant mental development: a meta-analytical review. *Alcohol Alcohol*, 38: 295–304. PMID:12814894

Theruvathu JA, Jaruga P, Nath RG *et al.* (2005). Polyamines stimulate the formation of mutagenic 1,N2-propanodeoxyguanosine adducts from acetaldehyde. *Nucleic Acids Res*, 33: 3513–3520. doi:10.1093/nar/gki661 PMID:15972793

Thomasson HR, Beard JD, Li T-K (1995). ADH2 gene polymorphisms are determinants of alcohol pharmacokinetics. *Alcohol Clin Exp Res*, 19: 1494–1499. doi:10.1111/j.1530-0277.1995.tb01013.x PMID:8749816

Til HP, Woutersen RA, Feron VJ, Clary JJ (1988). Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. *Food Chem Toxicol*, 26: 447–452. doi:10.1016/0278-6915(88)90056-7 PMID:3391468

Tillonen J, Kaihovaara P, Jousimies-Somer H *et al.* (1998). Role of catalase in in vitro acetaldehyde formation by human colonic contents. *Alcohol Clin Exp Res*, 22: 1113–1119. PMID:9726283

Tindberg N (2003). Phorbol ester induces CYP2E1 in astrocytes, through a protein kinase C- and tyrosine kinase-dependent mechanism. *J Neurochem*, 86: 888–895. doi:10.1046/j.1471-4159.2003.01897.x PMID:12887687

Tindberg N & Ingelman-Sundberg M (1996). Expression, catalytic activity, and inducibility of cytochrome P450 2E1 (CYP2E1) in the rat central nervous system. *J Neurochem*, 67: 2066–2073. doi:10.1046/j.1471-4159.1996.67052066.x PMID:8863515

Tomera JF, Skipper PL, Wishnok JS *et al.* (1984). Inhibition of N-nitrosodimethylamine metabolism by ethanol and other inhibitors in the isolated perfused rat liver. *Carcinogenesis*, 5: 113–116. doi:10.1093/carcin/5.1.113 PMID:6690081

Torres-Bezauri2002)

Tranah GJ, Bugni J, Giovannucci E *et al.* (2006). O6-Methylguanine–DNA methyltransferase Leu84Phe and Ile143Val polymorphisms and risk of colorectal cancer in the Nurses' Health Study and Physicians' Health Study (United States). *Cancer Causes Control*, 17: 721–731. doi:10.1007/s10552-006-0005-y PMID:16633920

Triano EA, Slusher LB, Atkins TA *et al.* (2003). Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. *Cancer Res*, 63: 3092–3100. PMID:12810634

Trinh BN, Ong C-N, Coetzee GA *et al.* (2002). Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. *Hum Genet*, 111: 299–302. doi:10.1007/s00439-002-0779-2 PMID:12215845

Tsukamoto H, French SW, Reidelberger RD, Largman C (1985). Cyclical pattern of blood alcohol levels during continuous intragastric ethanol infusion in rats. *Alcohol Clin Exp Res*, 9: 31–37. doi:10.1111/j.1530-0277.1985.tb05046.x PMID:3887966

Tsukamoto H, Horne W, Kamimura S *et al.* (1995). Experimental liver cirrhosis induced by alcohol and iron. *J Clin Invest*, 96: 620–630. doi:10.1172/JCI118077 PMID:7615836

Tsumura K, Hayashi T, Suematsu C *et al.* (1999). Daily alcohol consumption and the risk of type 2 diabetes in Japanese men: the Osaka Health Survey. *Diabetes Care*, 22: 1432–1437. doi:10.2337/diacare.22.9.1432 PMID:10480505

Tsutsumi M, Lasker JM, Shimizu M *et al.* (1989). The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. *Hepatology*, 10: 437–446. doi:10.1002/hep.1840100407 PMID:2673969

Tsutsumi M, Lasker JM, Takahashi T, Lieber CS (1993). In vivo induction of hepatic P4502E1 by ethanol: role of increased enzyme synthesis. *Arch Biochem Biophys*, 304: 209–218. doi:10.1006/abbi.1993.1341 PMID:8323286

Tsutsumi M, Wang J-S, Takase S, Takada A (1994). Hepatic messenger RNA contents of cytochrome P4502E1 in patients with different P4502E1 genotypes. *Alcohol Alcohol Suppl*, 29: 129–32. PMID:9063815

Tuma DJ, Smith SL, Sorrell MF (1991). Acetaldehyde and microtubules. *Ann N Y Acad Sci*, 625: Suppl 1786–792. doi:10.1111/j.1749-6632.1991.tb33920.x PMID:2058934

Tuyns AJ (2001). Alcohol and cancer *Pathol Biol (Paris)*, 49: 759–763. PMID:11762139

Uematsu F, Kikuchi H, Motomiya M *et al.* (1991). Association between restriction fragment length polymorphism of the human cytochrome P450IIE1 gene and susceptibility to lung cancer. *Jpn J Cancer Res*, 82: 254–256. PMID:1673675

Ulrich CM, Bigler J, Bostick R *et al.* (2002). Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. *Cancer Res*, 62: 3361–3364. PMID:12067974

Ulrich CM, Curtin K, Potter JD *et al.* (2005). Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*, 14: 2509–2516. doi:10.1158/1055-9965.EPI-05-0261 PMID:16284371

Ulrich CM, Kampman E, Bigler J *et al.* (2000). Lack of association between the C677T MTHFR polymorphism and colorectal hyperplastic polyps. *Cancer Epidemiol Biomarkers Prev*, 9: 427–433. PMID:10794488

Ulvik A, Evensen ET, Lien EA *et al.* (2001). Smoking, folate and methylenetetrahydrofolate reductase status as interactive determinants of adenomatous and hyperplastic polyps of colorectum. *Am J Med Genet*, 101: 246–254. doi:10.1002/ajmg.1370 PMID:11424140

Ulvik A, Vollset SE, Hansen S *et al.* (2004). Colorectal cancer and the methylenetetrahydrofolate reductase 677C -> T and methionine synthase 2756A → G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. *Cancer Epidemiol Biomarkers Prev*, 13: 2175–2180. PMID:15598777

Umulis DM, Gürmen NM, Singh P, Fogler HS (2005). A physiologically based model for ethanol and acetaldehyde metabolism in human beings. *Alcohol*, 35: 3–12. doi:10.1016/j.alcohol.2004.11.004 PMID:15922132

Upton DC, Wang X, Blans P *et al.* (2006). Mutagenesis by exocyclic alkylamino purine adducts in Escherichia coli. *Mutat Res*, 599: 1–10. PMID:16488449

Vaca CE, Fang JL, Schweda EK (1995). Studies of the reaction of acetaldehyde with deoxynucleosides. *Chem Biol Interact*, 98: 51–67. doi:10.1016/0009-2797(95)03632-V PMID:7586051

Väkeväinen S, Tillonen J, Agarwal DP *et al.* (2000). High salivary acetaldehyde after a moderate dose of alcohol in ALDH2-deficient subjects: strong evidence for the local carcinogenic action of acetaldehyde. *Alcohol Clin Exp Res*, 24: 873–877. doi:10.1111/j.1530-0277.2000.tb02068.x PMID:10888077

Väkeväinen S, Tillonen J, Salaspuro M (2001). 4-Methylpyrazole decreases salivary acetaldehyde levels in ALDH2-deficient subjects but not in subjects with nor-

mal ALDH2. *Alcohol Clin Exp Res*, 25: 829–834. doi:10.1111/j.1530-0277.2001.tb02286.x PMID:11410717

Välimäki MJ, Härkönen M, Eriksson CJ, Ylikahri RH (1984). Sex hormones and adrenocortical steroids in men acutely intoxicated with ethanol. *Alcohol*, 1: 89–93. doi:10.1016/0741-8329(84)90043-0 PMID:6443186

Välimäki M, Tuominen JA, Huhtaniemi I, Ylikahri R (1990). The pulsatile secretion of gonadotropins and growth hormone, and the biological activity of luteinizing hormone in men acutely intoxicated with ethanol. *Alcohol Clin Exp Res*, 14: 928–931.. doi:10.1111/j.1530-0277.1990.tb01840.x PMID:2128439

van der Put NMJ, Gabreëls F, Stevens EMB *et al.* (1998). A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet*, 62: 1044–1051. doi:10.1086/301825 PMID:9545395

van der Put NMJ, van der Molen EF, Kluijtmans LAJ *et al.* (1997). Sequence analysis of the coding region of human methionine synthase: relevance to hyperhomocysteinaemia in neural-tube defects and vascular disease. *QJM*, 90: 511–517. doi:10.1093/qjmed/90.8.511 PMID:9327029

van Zeeland AA, de Groot AJ, Hall J, Donato F (1999). 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutat Res*, 439: 249–257. PMID:10023075

Vasiliou V, Pappa A, Estey T (2004). Role of human aldehyde dehydrogenases in endobiotic and xenobiotic metabolism. *Drug Metab Rev*, 36: 279–299. doi:10.1081/DMR-120034001 PMID:15237855

Vasiliou V, Pappa A, Petersen DR (2000). Role of aldehyde dehydrogenases in endogenous and xenobiotic metabolism. *Chem Biol Interact*, 129: 1–19. doi:10.1016/S0009-2797(00)00211-8 PMID:11154732

Vasiliou V, Reuter SF, Kozak CA, Nebert DW (1993). Mouse dioxin-inducible cytosolic aldehyde dehydrogenase-3: AHD4 cDNA sequence, genetic mapping, and differences in mRNA levels. *Pharmacogenetics*, 3: 281–290. doi:10.1097/00008571-199312000-00002 PMID:8148869

Vasiliou V, Ziegler TL, Bludeau P *et al.* (2006). CYP2E1 and catalase influence ethanol sensitivity in the central nervous system. *Pharmacogenet Genomics*, 16: 51–58. doi:10.1097/01.fpc.0000182777.95555.56 PMID:16344722

Verschueren K (1983) Handbook of Environmental Data of Organic Chemicals, 2nd ed., Van Nostrand Reinhold Co., New York, NY, p.141.

Visapää JP, Götte K, Benesova M *et al.* (2004). Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. *Gut*, 53: 871–876. doi:10.1136/gut.2003.018994 PMID:15138216

Waddell WJ, Marlowe C, Pierce WM Jr (1987). Inhibition of the localization of urethane in mouse tissues by ethanol. *Food Chem Toxicol*, 25: 527–531. doi:10.1016/0278-6915(87)90204-3 PMID:3623342

Wang D, Ritchie JM, Smith EM *et al.* (2005a). Alcohol dehydrogenase 3 and risk of squamous cell carcinomas of the head and neck. *Cancer Epidemiol Biomarkers Prev*, 14: 626–632. doi:10.1158/1055-9965.EPI-04-0343 PMID:15767341

Wang G-X, Wang B-Y, Liu C-R (2002). The relationship between activities of hepatic and gastric alcohol dehydrogenase and occurrence of chronic alcoholic liver disease. *Hepatobiliary Pancreat Dis Int*, 1: 406–410. PMID:14607716

Wang L, Miao X, Tan W *et al.* (2005b). Genetic polymorphisms in methylenetetrahydrofolate reductase and thymidylate synthase and risk of pancreatic cancer. *Clin Gastroenterol Hepatol*, 3: 743–751. doi:10.1016/S1542-3565(05)00156-4 PMID:16234002

Wang M, McIntee EJ, Cheng G *et al.* (2000). Identification of DNA adducts of acetaldehyde. *Chem Res Toxicol*, 13: 1149–1157. doi:10.1021/tx000118t PMID:11087437

Wang M, Yu N, Chen L *et al.* (2006). Identification of an acetaldehyde adduct in human liver DNA and quantitation as N2-ethyldeoxyguanosine. *Chem Res Toxicol*, 19: 319–324. doi:10.1021/tx0502948 PMID:16485909

Wang XD (2005). Alcohol, vitamin A, and cancer. *Alcohol*, 35: 251–258. doi:10.1016/j.alcohol.2005.04.006 PMID:16054987

Wangenheim J & Bolcsfoldi G (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis*, 3: 193–205. doi:10.1093/mutage/3.3.193 PMID:3045481

Watanabe J, Hayashi S-I, Kawajiri K (1994). Different regulation and expression of the human CYP2E1 gene due to the RsaI polymorphism in the 5′-flanking region. *J Biochem*, 116: 321–326. PMID:7529759

Watanabe M (1997). Immunohistochemical localization of alcohol dehydrogenase (ADH) in the stomach before and after abstinence of alcohol in alcoholics–using confocal laser scanning microscopy. *Kurume Med J*, 44: 263–272. PMID:9476469

Watson RR, Odeleye OE, Eskelson CD, Mufti SI (1992). Alcohol stimulation of lipid peroxidation and esophageal tumor growth in mice immunocompromised by retrovirus infection. *Alcohol*, 9: 495–500. doi:10.1016/0741-8329(92)90086-P PMID:1335272

Webster WS, Walsh DA, McEwen SE, Lipson AH (1983). Some teratogenic properties of ethanol and acetaldehyde in C57BL/6J mice: implications for the study of the fetal alcohol syndrome. *Teratology*, 27: 231–243. doi:10.1002/tera.1420270211 PMID:6867945

Wedel M, Pieters JE, Pikaar NA, Ockhuizen Th (1991). Application of a three-compartment model to a study of the effects of sex, alcohol dose and concentration, exercise and food consumption on the pharmacokinetics of ethanol in healthy volunteers. *Alcohol Alcohol*, 26: 329–336. PMID:1930365

Wegener M, Schaffstein J, Dilger U *et al.* (1991). Gastrointestinal transit of solid-liquid meal in chronic alcoholics. *Dig Dis Sci*, 36: 917–923. doi:10.1007/BF01297141 PMID:2070705

Weisberg I, Tran P, Christensen B *et al.* (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*, 64: 169–172. doi:10.1006/mgme.1998.2714 PMID:9719624

Wheeler MD, Kono H, Yin M *et al.* (2001a). The role of Kupffer cell oxidant production in early ethanol-induced liver disease. *Free Radic Biol Med*, 31: 1544–1549. doi:10.1016/S0891-5849(01)00748-1 PMID:11744328

Wheeler MD, Kono H, Yin M *et al.* (2001b). Delivery of the Cu/Zn-superoxide dismutase gene with adenovirus reduces early alcohol-induced liver injury in rats. *Gastroenterology*, 120: 1241–1250. doi:10.1053/gast.2001.23253 PMID:11266387

Whitfield JB, Nightingale BN, Bucholz KK *et al.* (1998). ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res*, 22: 1463–1469. PMID:9802529

Wilkin JK & Fortner G (1985a). Cutaneous vascular sensitivity to lower aliphatic alcohols and aldehydes in Orientals. *Alcohol Clin Exp Res*, 9: 522–525. doi:10.1111/j.1530-0277.1985.tb05596.x PMID:2936266

Wilkin JK & Fortner G (1985b). Ethnic contact urticaria to alcohol. *Contact Dermatitis*, 12: 118–120. doi:10.1111/j.1600-0536.1985.tb01073.x PMID:3987255

Wilson DM 3rd, Tentler JJ, Carney JP *et al.* (1994). Acute ethanol exposure suppresses the repair of O6-methylguanine DNA lesions in castrated adult male rats. *Alcohol Clin Exp Res*, 18: 1267–1271. doi:10.1111/j.1530-0277.1994.tb00117.x PMID:7847618

Wolff PH (1972). Ethnic differences in alcohol sensitivity. *Science*, 175: 449–450. doi:10.1126/science.175.4020.449 PMID:5007912

Wong NACS, Rae F, Simpson KJ *et al.* (2000). Genetic polymorphisms of cytochrome P4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis. *Mol Pathol*, 53: 88–93. doi:10.1136/mp.53.2.88 PMID:10889908

Woodcroft KJ, Hafner MS, Novak RF (2002). Insulin signaling in the transcriptional and posttranscriptional regulation of CYP2E1 expression. *Hepatology*, 35: 263–273. doi:10.1053/jhep.2002.30691 PMID:11826398

Woodruff RC, Mason JM, Valencia R, Zimmering S (1985). Chemical mutagenesis testing in Drosophila. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ Mutagen*, 7: 677–702. doi:10.1002/em.2860070507 PMID:3930237

Woutersen RA, Appelman LM, Feron VJ *et al.* (1984). Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: interim results after 15 months. *Toxicology*, 31: 123–133. doi:10.1016/0300-483X(84)90004-0 PMID:6740689

Woutersen RA, Appelman LM, Van Garderen-Hoetmer A *et al.* (1986). Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology*, 41: 213–231. doi:10.1016/0300-483X(86)90201-5 PMID:3764943

Woutersen RA & Feron VJ (1987). Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. *Toxicology*, 47: 295–305. doi:10.1016/0300-483X(87)90059-X PMID:3424385

Wu C-F, Wu D-C, Hsu H-K *et al.* (2005). Relationship between genetic polymorphisms of alcohol and aldehyde dehydrogenases and esophageal squamous cell carcinoma risk in males. *World J Gastroenterol*, 11: 5103–5108. PMID:16127737

Wu Y-S, Salmela KS, Lieber CS (1998). Microsomal acetaldehyde oxidation is negligible in the presence of ethanol. *Alcohol Clin Exp Res*, 22: 1165–1169. PMID:9726291

Xiao Q, Weiner H, Crabb DW (1996). The mutation in the mitochondrial aldehyde dehydrogenase (ALDH2) gene responsible for alcohol-induced flushing increases turnover of the enzyme tetramers in a dominant fashion. *J Clin Invest*, 98: 2027–2032. doi:10.1172/JCI119007 PMID:8903321

Xiao Q, Weiner H, Johnston T, Crabb DW (1995). The aldehyde dehydrogenase ALDH2*2 allele exhibits dominance over ALDH2*1 in transduced HeLa cells. *J Clin Invest*, 96: 2180–2186. doi:10.1172/JCI118272 PMID:7593603

Yamada Y, Weller RO, Kleihues P, Ludeke BI (1992). Effects of ethanol and various alcoholic beverages on the formation of O6-methyldeoxyguanosine from concurrently administered N-nitrosomethylbenzylamine in rats: a dose–response study. *Carcinogenesis*, 13: 1171–1175. doi:10.1093/carcin/13.7.1171 PMID:1638683

Yamagishi Y, Horie Y, Kajihara M *et al.* (2004). Hepatocellular carcinoma in heavy drinkers with negative markers for viral hepatitis. *Hepatol Res*, 28: 177–183. doi:10.1016/j.hepres.2003.11.009 PMID:15040957

Yamamoto T, Pierce WM Jr, Hurst HE *et al.* (1988). Inhibition of the metabolism of urethane by ethanol. *Drug Metab Dispos*, 16: 355–358. PMID:2900725

Yanagawa Y, Chen JC, Hsu LC, Yoshida A (1995). The transcriptional regulation of human aldehyde dehydrogenase I gene. The structural and functional analysis of the promoter. *J Biol Chem*, 270: 17521–17527. PMID:7615557

Yang B, O'Reilly DA, Demaine AG, Kingsnorth AN (2001). Study of polymorphisms in the CYP2E1 gene in patients with alcoholic pancreatitis. *Alcohol*, 23: 91–97. doi:10.1016/S0741-8329(00)00135-X PMID:11331106

Yang CS, Patten CJ, Ishizaki H, Yoo J-SH (1991). Induction, purification, and characterization of cytochrome P450IIE. *Methods Enzymol*, 206: 595–603. doi:10.1016/0076-6879(91)06129-Q PMID:1664482

Yang C-X, Matsuo K, Ito H *et al.* (2005). Esophageal cancer risk by ALDH2 and ADH2 polymorphisms and alcohol consumption: exploration of gene-environment and gene-gene interactions. *Asian Pac J Cancer Prev*, 6: 256–262. PMID:16235983

Yao C-T, Liao C-S, Yin S-J (1997). Human hepatic alcohol and aldehyde dehydrogenases: genetic polymorphism and activities. *Proc Natl Sci Counc Repub China B*, 21: 106–111. PMID:9309874

Yasunami M, Chen C-S, Yoshida A (1991). A human alcohol dehydrogenase gene (ADH6) encoding an additional class of isozyme. *Proc Natl Acad Sci U S A*, 88: 7610–7614. doi:10.1073/pnas.88.17.7610 PMID:1881901

Yin G, Kono S, Toyomura K *et al.* (2004). Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Sci*, 95: 908–913. doi:10.1111/j.1349-7006.2004.tb02201.x PMID:15546509

Yin S-J, Chou F-J, Chao S-F *et al.* (1993). Alcohol and aldehyde dehydrogenases in human esophagus: comparison with the stomach enzyme activities. *Alcohol Clin Exp Res*, 17: 376–381. doi:10.1111/j.1530-0277.1993.tb00779.x PMID:8488982

Yin S-J, Liao C-S, Lee Y-C *et al.* (1994). Genetic polymorphism and activities of human colon alcohol and aldehyde dehydrogenases: no gender and age differences. *Alcohol Clin Exp Res*, 18: 1256–1260. doi:10.1111/j.1530-0277.1994.tb00115.x PMID:7847616

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*, 112: 766–775. doi:10.1053/gast.1997.v112.pm9041238 PMID:9041238

Yokoyama A, Kato H, Yokoyama T *et al.* (2002b). Genetic polymorphisms of alcohol and aldehyde dehydrogenases and glutathione S-transferase M1 and drinking, smoking, and diet in Japanese men with esophageal squamous cell carcinoma. *Carcinogenesis*, 23: 1851–1859. doi:10.1093/carcin/23.11.1851 PMID:12419833

Yokoyama A, Kato H, Yokoyama T *et al.* (2006a). Esophageal squamous cell carcinoma and aldehyde dehydrogenase-2 genotypes in Japanese females. *Alcohol Clin Exp Res*, 30: 491–500. doi:10.1111/j.1530-0277.2006.00053.x PMID:16499490

Yokoyama A, Muramatsu T, Ohmori T *et al.* (1996). Esophageal cancer and aldehyde dehydrogenase-2 genotypes in Japanese males. *Cancer Epidemiol Biomarkers Prev*, 5: 99–102. PMID:8850269

Yokoyama A, Muramatsu T, Ohmori T *et al.* (1998a). Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis*, 19: 1383–1387. doi:10.1093/carcin/19.8.1383 PMID:9744533

Yokoyama A, Muramatsu T, Omori T *et al.* (2001). Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. *Carcinogenesis*, 22: 433–439. doi:10.1093/carcin/22.3.433 PMID:11238183

Yokoyama A, Ohmori T, Muramatsu T *et al.* (1998b). Short-term follow-up after endoscopic mucosectomy of early esophageal cancer and aldehyde dehydrogenase-2 genotype in Japanese alcoholics. *Cancer Epidemiol Biomarkers Prev*, 7: 473–476. PMID:9641490

Yokoyama A & Omori T (2003). Genetic polymorphisms of alcohol and aldehyde dehydrogenases and risk for esophageal and head and neck cancers. *Jpn J Clin Oncol*, 33: 111–121. doi:10.1093/jjco/hyg026 PMID:12672787

Yokoyama A & Omori T (2005). Genetic polymorphisms of alcohol and aldehyde dehydrogenases and risk for esophageal and head and neck cancers. *Alcohol*, 35: 175–185. doi:10.1016/j.alcohol.2005.04.003 PMID:16054979

Yokoyama A, Omori T, Yokoyama T *et al.* (2006b). Risk of squamous cell carcinoma of the upper aerodigestive tract in cancer-free alcoholic Japanese men: an endoscopic follow-up study. *Cancer Epidemiol Biomarkers Prev*, 15: 2209–2215. doi:10.1158/1055-9965.EPI-06-0435 PMID:17119048

Yokoyama A, Watanabe H, Fukuda H *et al.* (2002a). Multiple cancers associated with esophageal and oropharyngolaryngeal squamous cell carcinoma and the aldehyde dehydrogenase-2 genotype in male Japanese drinkers. *Cancer Epidemiol Biomarkers Prev*, 11: 895–900. PMID:12223435

Yokoyama H, Baraona E, Lieber CS (1995). Upstream structure of human ADH7 gene and the organ distribution of its expression. *Biochem Biophys Res Commun*, 216: 216–222. doi:10.1006/bbrc.1995.2613 PMID:7488092

Yokoyama T, Saito K, Lwin H *et al.* (2005). Epidemiological evidence that acetaldehyde plays a significant role in the development of decreased serum folate concentration and elevated mean corpuscular volume in alcohol drinkers. *Alcohol Clin Exp Res*, 29: 622–630. doi:10.1097/01.ALC.0000158842.24218.03 PMID:15834228

Yokoyama T, Yokoyama A, Kato H *et al.* (2003). Alcohol flushing, alcohol and aldehyde dehydrogenase genotypes, and risk for esophageal squamous cell carcinoma in Japanese men. *Cancer Epidemiol Biomarkers Prev*, 12: 1227–1233. PMID:14652286

Yoo JS, Ning SM, Pantuck CB *et al.* (1991). Regulation of hepatic microsomal cytochrome P450IIE1 level by dietary lipids and carbohydrates in rats. *J Nutr*, 121: 959–965. PMID:2051238

Yoshida A, Huang I-Y, Ikawa M (1984). Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc Natl Acad Sci U S A*, 81: 258–261. doi:10.1073/pnas.81.1.258 PMID:6582480

Yu H & Berkel J (1999). Do insulin-like growth factors mediate the effect of alcohol on breast cancer risk? *Med Hypotheses*, 52: 491–496. doi:10.1054/mehy.1998.0828 PMID:10459827

Yu H & Rohan T (2000). Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst*, 92: 1472–1489. doi:10.1093/jnci/92.18.1472 PMID:10995803

Yu M-W, Gladek-Yarborough A, Chiamprasert S *et al.* (1995). Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroenterology*, 109: 1266–1273. doi:10.1016/0016-5085(95)90587-1 PMID:7557094

Yu SZ, Huang XE, Koide T *et al.* (2002). Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China. *Jpn J Cancer Res*, 93: 1287–1292. PMID:12495467

Zavras AI, Wu T, Laskaris G *et al.* (2002). Interaction between a single nucleotide polymorphism in the alcohol dehydrogenase 3 gene, alcohol consumption and oral cancer risk. *Int J Cancer*, 97: 526–530. doi:10.1002/ijc.1642 PMID:11802217

Zeiger E, Anderson B, Haworth S *et al.* (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ Mol Mutagen*, 19: Suppl 212–141. doi:10.1002/em.2850190603 PMID:1541260

Zerilli A, Lucas D, Amet Y *et al.* (1995). Cytochrome P-450 2E1 in rat liver, kidney and lung microsomes after chronic administration of ethanol either orally or by inhalation. *Alcohol Alcohol*, 30: 357–365. PMID:7545990

Zhang ZL, Yang J, Zhang QA, Cao XS (1991). Studies on the utilization of a plant SCE test in detecting potential mutagenic agents. *Mutat Res*, 261: 69–73. doi:10.1016/0165-1218(91)90099-8 PMID:1881408

Zintzaras E, Stefanidis I, Santos M, Vidal F (2006). Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? *Hepatology*, 43: 352–361. doi:10.1002/hep.21023 PMID:16440362