Individual susceptibility and alcohol effects: biochemical and genetic aspects

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Summary. The large interethnic and interindividual variability in alcohol-induced toxic effects comes from a combination of genetic and environmental factors, influencing ethanol toxicokinetics. The hepatic enzymatic systems involved in ethanol metabolism are alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and microsomal P4502E1 (CYP2E1). ADH oxidizes ethanol to acetaldehyde, which is very efficiently oxidized to acetate by ALDH. About 10% of moderate quantities of ethanol is metabolised by CYP2E1; the percentage increases when ADH is saturated. During ethanol metabolism reactive oxygen species and hydroxyethyl radicals are generated, causing oxidative stress, responsible for most ethanol-induced liver damage. For their critical role in detoxifying radicals, glutathione S-transferase are gaining attention in the etiology of alcoholism. All these enzymes have been shown to be polymorphic, giving rise to altered phenotypes. For this reason recent studies have looked for a correlation between metabolic variability and differences in alcohol abuse-related effects.

Key words: ethanol, interindividual variability, polymorphism, alcohol dehydrogenase, aldehyde dehydrogenase, CYP2E1, glutathione S-transferase.

Riassunto *(Variabilità individuale ed effetti dell'alcol: aspetti biochimici e genetici)*. L'ampia variabilità interetnica ed interindividuale degli effetti tossici dovuti all'alcol deriva da una combinazione di fattori genetici ed ambientali che influenzano la tossicocinetica dell'etanolo. I sistemi enzimatici epatici coinvolti nel metabolismo dell'etanolo sono l'alcol deidrogenasi (ADH), l'aldeide deidrogenasi (ALDH) e il citocromo P450 2E1 (CYP2E1). L'ADH ossida l'etanolo ad acetaldeide che è efficientemente biotrasformata ad acetato dalla ALDH. Circa il 10% di moderate quantità di etanolo viene metabolizzato dal CYP2E1; la percentuale aumenta quando l'ADH è saturata. Durante il metabolismo dell'etanolo si generano specie reattive dell'ossigeno e radicali idrossietilici che causano stress ossidativo, principale responsabile del danno epatico indotto da etanolo. A causa del ruolo critico delle glutatione S-transferasi nella detossificazione dei radicali, il loro coinvolgimento nell'eziologia dell'alcolismo sta ricevendo crescente attenzione. Tutti questi enzimi sono presenti come forme polimorfiche che possono originare fenotipi alterati. Per questa ragione studi recenti hanno cercato una correlazione fra variabilità metabolica e differenze negli effetti dell'abuso di alcol.

Parole chiave: etanolo, variabilità interindividuale, polimorfismo, alcol deidrogenasi, aldeide deidrogenasi, CYP2E1, glutatione S-transferasi.

INTRODUCTION

Alcoholism is a common disorder with a complex origin and outcome, since individuals react differently when exposed to comparable amounts of alcohol. Many epidemiological, biomedical and psychosocial studies support the hypothesis that some individuals suffer more severe adverse effects following alcohol use. Physiological features (such as age and gender) and socio-cultural/psychological factors may play a relevant role in determining the huge interindividual variability in the thresholds and lifetime prevalence of this disease. Indeed, social restrictions have been shown to have a huge influence on the risk for alcohol dependence, particularly in societies with a high prevalence of alcoholism [1]. Excessive and prolonged use of alcoholic beverages is the cause of serious social and medical disorders in a significant number of individuals associated with socioeconomic consequences for the rest of the population. Alcohol related pathologies are very often related to the deficient nutritional status of chronic drinkers, due to unbalanced diet and ethanol interference in the uptake and utilization of carbohydrates, lipids and vitamins, particularly vitamin A. Indeed, ethanol has been shown to inhibit the oxidation of retinol to retinoic acid (the active form of vitamin A) by competing for alcohol dehydrogenases [2]. As a consequence, the levels of retinoic acid, which is essential for growth and maintenance of normal epithelial function, are decreased in alcoholics. Ethanol has also been demonstrated to have teratogenic potential: alcohol consumption during pregnancy can potentially result in effects in the foetus ranging

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from transient outcome to a quite severe neurologic disorder known as foetal alcohol syndrome (FAS). The syndrome has been associated to both ethanol metabolism and related oxidative stress and to reduction in retinoic acid production during gestation.

Family studies on twins and adoptee estimated that individual risk for alcoholism can be equally addressed to environmental and genetic factors which show a high degree of interaction [3].

Ethnicity seems also to confer different susceptibility to ethanol toxicity, as suggested by studies showing that, when compared with African Americans or native Americans, Caucasians have higher and lower rates of ethanol elimination, respectively. Differences in liver mass may only partially explain ethnic and gender differences measured in alcohol clearance [4]. Indeed, recent molecular genetic research has assigned to functional polymorphisms at those genes encoding enzymes involved in ethanol toxicokinetics, the pivotal role in determining the differential susceptibility in alcohol-induced toxic effects. This genetic features may act in combination with environmental factors, such as nutrition, life-style and exposure to other xenobiotics, responsible for the acquired modulation (induction/inhibition) of the same enzymatic activities [5].

In the paper the main features of ethanol metabolism and the polymorphisms of the most relevant enzymes involved in determining the eventual different susceptibility to alcohol-induced effect will be briefly presented.

Ethanol toxicokinetics and metabolism

Ethanol-induced effects are due to both ethanol *per se* and to the products of its metabolism, including redox changes related to the production of acetaldehyde and acetate.

The time course of ethanol blood concentration after ingestion of alcoholic drinks is strictly dependent on its toxicokinetics, which determines the dose to the target organs and the toxicodynamic responses to ethanol [6].

After oral administration, ethanol is readily absorbed by the gastrointestinal tract; absorption takes place by passive diffusion through the stomach wall (about 20%), being the remaining 80% absorbed through the duodenum and small intestine wall [5]. The rate of absorption varies with the time of the day, the dosage form, the concentration of ethanol and the drinking pattern, mainly related to the gastric emptying status. After oral absorption of ingested doses <0.3 g/kg, the removal of ethanol by the liver, before it reaches systemically other organs (hepatic first-pass effect), is pronounced. At higher ethanol doses, this effect is not easy to be quantitatively defined, also due to the great interindividual variation in the percentage of absorbed dose. Once in the bloodstream, ethanol is uniformly distributed in the body water space; indeed, being highly water soluble, it does not need to bind to plasma proteins and therefore there is a strong correlation between ethanol volume of distribution and total body water [5].

Elimination of absorbed ethanol occurs primarily through metabolism (95-98%), with small fractions of the administered dose being excreted unchanged in the breath (0.7%), sweat (0.1%), and urine (0.3%) [5, 7].

In adult nonalcoholic individuals, most ethanol biotransformation occurs in the liver *(Fig. 1)* mainly *via* oxidation catalyzed by alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and by a cytochrome P450 isoform (CYP2E1) [8, 9].

In the cytosol of hepatocytes, ethanol is oxidized to acetaldehyde, in a reversible reaction catalyzed by class I ADH, a high affinity (K_m= 0.05-0.1 g/l) and low capacity enzyme, becoming saturated after only few drinks. Acetaldehyde is then oxidized in a non-reversible reaction to acetate, by the mithocondrial form of ALDH. Since the enzyme has a very low K_m, the elimination of acetaldehyde is very efficient, so that the product of ethanol oxidation, which is highly toxic, is eliminated soon after its formation. It has been estimated that during ethanol intoxication only 1-2% of the acetaldehyde formed in the liver enter the bloodstream, giving rise to negligible peripheral venous levels (≈1 μmol/l) [10]. The activated form of acetate, acetyl CoA, may be further metabolized leading to ketone bodies, amino acids, fatty acids and steroids [8]; when it is oxidized in the Krebs cycle, CO_2 and water are formed as the end-products of ethanol oxidation. Both ADH and ALDH utilize as the cofactor NAD^+, which is reduced to NADH *(Fig. 1)*: as a consequence, during ethanol oxidation the ratio NADH/NAD^+ is significantly increased, altering the cellular

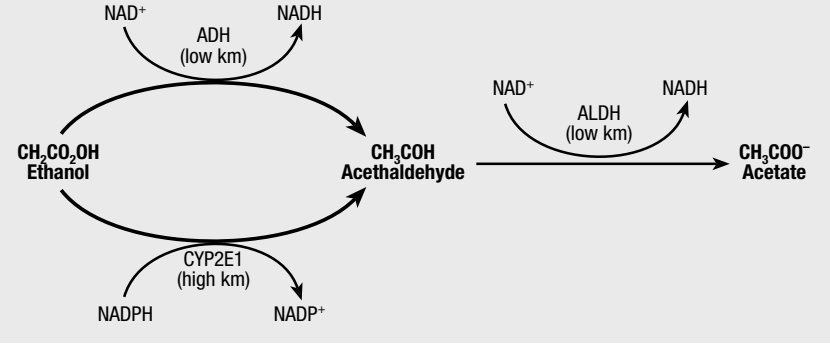

Fig. 1 | *Pathways of ethanol metabolism in the liver. ALDH=acetaldehyde dehydrogenase; NAD^+=nicotinamide adenine dinucleotide; NADH= reduced NAD^+; $NADP^+$=nicotinamide adenine dinucleotide phosphate; NADPH=reduced $NADP^+$; ADH = alcohol dehydrogenase; CYP2E1 = cytochrome P-450 isoform 2E1.*

Table 1 | *Polymorphisms of alcohol dehydrogenase (ADH) genes*

Class (Protein)	Gene	Subunit	Nucleotide change	Effect	Chromosomal location
Class I ADH					
ADH1	ADH1*1	α		wild-type	4q22
ADH2	ADH2*1	β1		wild-type	4q22
	ADH*2	β2	47G > A	His^{47} ; increased V_{max}	
	ADH2*3	β3	369C > T	Cys^{369}; increased V_{max}	
ADH3	ADH3*1	γ1		wild-type	4q22
	ADH3*2	γ2	271C > T; 349G > A	Gln^{271};Val^{349}	
Class II ADH					
ADH4	ADH4	π	192T > A ; 159G > A; 75A >C	altered expression	4q21-25
Class III ADH					
ADH5	ADH5	χ		wild-type	4q21-25
Class IV ADH					
ADH7	ADH7	σ		wild-type	4q23-24
Class V ADH					
ADH6	ADH6	?§		?§	4q21-25

*§Subunit composition not known. *Adapted from [7].*

redox state and triggering a number of adverse effects, related to alcohol consumption [5]. The hepatic NADH re-oxidation seems to be the rate limiting step of the process and together with the functional ADH and ALDH activities regulate the steady-state of ethanol oxidation rate.

When a moderate dose of ethanol is ingested, a small but significant amount (≈10%) is metabolized by the microsomal NADPH dependent-oxidation catalyzed by CYP2E1 [8, 9]. This enzyme is characterized by a lower affinity (K_m= 0.5-0.8 g/l) with respect to ADH: its role in ethanol oxidation becomes relevant when large amounts of alcohol able to saturate ADH are ingested (>100 g per day). The capacity-limited elimination, due to CYP2E1 saturation, is counteracted by enzyme induction by ethanol itself, which is thus able to increase its own clearance in heavy drinkers and alcoholics.

Role of polymorphisms in ethanol metabolism and ethanol-induced effects

The important contribution of genetic factors to alcoholism is not explained by Mendelian inheritance of single genes, but there are strong evidences suggesting that this pathology is a genetically-influenced complex multifactorial disease. The increasing utilization of molecular technologies in the genetic research on alcoholism has focused the attention on the possible role of functional polymorphisms in genes encoding ethanol metabolizing enzymes. Those genetic variants produce enzymes with altered activity, changing the rate of toxic metabolites production or of their detoxication.

Thus, elucidation of the molecular mechanisms that control and influence elimination and metabolism of ethanol is important in understanding the biochemical basis of ethanol toxicity and alcohol abuse-related pharmacological and addictive consequences in humans. In addition, the identification of genetic polymorphisms and the characterization of their putative role in alcoholism vulnerability may help in improving prevention and treatment approach.

In the following the major genetic variants, giving rise to functional polymorphisms of enzymes involved both in ethanol metabolism (ADH, ALDH, CYP2E1) or in the onset of effects due to the alteration of the redox status (glutathione S-transferase enzymes), will be presented.

Alcohol dehydrogenase

Alcohol dehydrogenase (ADH) is a cytosolic enzyme able to metabolize ethanol and a wide variety of substrates, including other aliphatic alcohols, hydroxysteroids and lipid peroxidation products. ADH exists as a polygenic family of seven genes located on chromosome 4, translated in various human ADH forms *(Table 1)*. They can be divided into five major classes or distinct groups (I-V), according to their subunit composition as well as their physicochemical properties [11].

Human ADH is a dimeric protein, resulting from the association of different subunits with a molecular weight of 40 kD each. Class I ADH (ADH1, ADH2 and ADH3) isoenzymes are formed by different combinations of subunits (α, β, γ), coded by genes from either the same locus or different loci. Homodimeric proteins are formed by identical subunits coded by the same locus, while heterodimeric proteins are formed by alleles coded from different loci (*e.g.*, αβ, αγ) or by different alleles at the same locus (*e.g.*, β1β2, γ1γ2).

Although 20 ADH isoenzymes are known, which vary in their catalytic properties, relevant functional polymorphism has been found only for the beta and gamma subunits forming ADH2 and ADH3 [12]. Up to now no allelic polymorphism has been reported in

Table 2 | *Polymorphism of aldehyde dehydrogenase (ALDH) genes*

Locus	Gene	Nucleotide change	Effect	Chromosomal location
ALDH1B1	ALDH1B1*1		wild-type	9p13
	ALDH1B1*2	257C > T	Ala^{69}	
	ALDH1B1*3	320T > G	Leu^{90}	
	ALDH1B1*4	183C > T	silent	
ALDH2	ALDH2*1		wild-type	12q24.2
	ALDH2*2	1510G > A	Lys^{487}	
	ALDH2*3	1486G > A	Lys^{479}	
	ALDH2*4	1464G > A	silent	
ALDH3A1	ALDH3A1*1		wild-type	17p11.2
	ALDH3A1*2	985C > G	Ala^{329}	
ALDH3A2	ALDH3A2*1		wild-type	17p11.2
	ALDH3A2*2	521T del	frameshit	
	ALDH3A2*3	808G del	frameshit	
	ALDH3A2*4	941 del 3bp, 941 ins 21bp	Gly^{314}, Ala^{315}, 6 aa ins	
	ALDH3A2*5	641G > A	Tyr^{214}	
	ALDH3A2*6	1297GA del	frameshit	
	ALDH3A2*7	1311 ins 5bp	frameshit	
	ALDH3A2*8	1297GA del	frameshit	
ALDH4A1	ALDH4A1*1		wild-type	1
	ALDH4A1*2	21G del	frameshit	
	ALDH4A1*3	1055C > T	Leu^{352}	
	ALDH4A1*4	47C > T	Leu^{16}	
	ALDH4A1*5	1563T ins	frameshit	
ALDH9A1	ALDH9A1*1		wild-type	1q22-q23
	ALDH9A1*2	344G > C	Ser^{115}	
	ALDH9A1*3	327C > T	silent	

*ins: insertion; del: deletion; bp: base pair; aa: amino acids. *Adapted from [7].*

human populations for the α-, π- and χ-subunits of ADH [8]. It has been suggested that the ADH variants may be involved in different attitude to alcoholism, since allele frequencies differ between alcoholics and controls [13].

The *ADH2* gene may be present as *ADH2*1*, *ADH2*2* and *ADH2*3* encoding for β1, β2, and β3 subunit, respectively, which differ by single nucleotide exchanges; however, the difference of a single amino acid determines in the protein quite different catalytic properties. The enzyme containing the β1 subunit has high affinity and low capacity for ethanol, whereas the β2 and the β3 forms show lower affinity and higher capacity: the V_{max} of β2 homodimers is around 40-fold higher than that of β1 homodimers. As a consequence, the activities related to β2 and β3 subunits are not highly capacity-limited by large amount of ethanol ingestion.

ADH3 (encoding the γ subunits) is also polymorphic; however, the functional meaning of these variants is limited, since the γ1 homodimers (encoded by *ADH3*1*) have an only two-fold higher V_{max} than the one measured for γ2 homodimers (encoded by *ADH3*2*) [14].

Different tissues show differentially measurable human *ADH* gene expression; liver contains a large amount of ADH (representing about 3% of total soluble proteins in the hepatocyte) and expresses the widest number of isozymes, mainly class I. ADH5 (χ-ADH) is ubiquitously expressed in all human tissues tested so far; ADH4 (π-ADH) is solely expressed in liver, while ADH7 (σ-ADH) is the only isoform expressed at low level in the liver [6], but present at significant amounts in gastrointestinal tissue, mainly in the gastric mucosa of Caucasian, but nearly absent in Asians [15]. Similarly, a low ADH activity has been demonstrated in the gastric mucosa of females of Caucasian origin [16]. This feature has been associated with the lower gastric first pass effect in the toxicokinetics of ethanol observed both in Asian populations and females, as well as with ethanol decreased clearance and consequently with increased alcohol blood levels which may contribute to the higher susceptibility of females to ethanol-induced effects.

The actual role of ADH in alcohol related pathologies has not been elucidated yet. It is not clear the mechanism according to which heavy drinkers with liver damage and other gastrointestinal disorders show

a higher serum ADH activity [17], in spite of an equal rate of ethanol metabolism with respect to controls. Although some correlation between ADH polymorphisms and ethanol susceptibility has been observed, the effect of ADH variants on the risk of alcoholic liver disease could be complex: high activity-ADH variants decrease alcoholism risk in carrying individuals, but if they persist in drinking, the risk for hepatic injury might increase, resulting from high intrahepatic concentrations of acetaldehyde [18].

The prevalence of the variant forms of ADH vary in different ethnic populations: 95% of Caucasians have the β1 enzyme form *(ADH2*1)*; the 90% Orientals have the β2 form *(ADH2*2)* which is present in Europe with frequency varying from 5–10% of the English up to 20% of the Swiss population; the β3 form *(ADH2*3)* is present in the 24% Africans and African-Americans [14]. Analogously for *ADH3*1* and *ADH3*2*: 90% of Asians and 50% of Caucasians have the γ1 and γ2 form, respectively [19]. In *Table 1*, the molecular basis of polymorphic changes in various ADH alleles and the effect on enzyme activity are summarized.

ALDEHYDE DEHYDROGENASES

Acetaldehyde, the toxic metabolite produced by enzymatic ethanol oxidation in the human liver, is further metabolized by ALDH in a NAD^+-dependent reaction. These enzymes have broad substrate specificity for aliphatic and aromatic aldehydes, which are irreversibly oxidized to their corresponding carboxylic acids. The ALDH are cytosolic enzymes, expressed in a wide range of tissues [8]. A number of isoenzymes of ALDH coded by different gene loci have been detected in humans, which differ in their electrophoretic mobility, kinetic properties, as well as in their cellular and tissue distribution and show a certain degree of overlapping substrate specificity.

Genes coding for ALDH enzymes are divided into nine major families *(Table 2)*; the major ones are family 1 corresponding to cytosolic ALDHs (ALDH1), family 2 to mitochondrial ALDHs (ALDH2), and family 3 which groups the major constitutive and inducible ALDH forms (ALDH3) found in human stomach, saliva, and hepatocarcinoma [8]. On the basis of kinetic properties and sequence similarities, the nomenclature for ALDH proteins has been recently revised; they have been tentatively classified as class 1 (low K_m, cytosolic), class 2 (low K_m, mitochondrial) and class 3 (high-K_m ALDH, such as those expressed in tumors, stomach and cornea) [18].

There are multiple molecular forms of ALDH in human liver, but only class I and class II isozymes, encoded by *ALDH1* and *ALDH2* genes, respectively, are thought to be involved in acetaldehyde oxidation [6].

Both ALDH1 and ALDH2 are homotetrameric enzymes, characterized by isoenzyme specific subunits (MW ≈54 kD each) and by different catalytic properties. The lowest K_m values for acetaldehyde has been measured for the mitochondrial ALDH2 (0.2-1 μM), although also ALDH1 has a relatively high affinity for the substrate (K_m value around 30 μM), consistently with their relevant involvement in ethanol oxidation process. ALDH3 and ALDH4 show a lower affinity towards both acetaldehyde and propionaldehyde as substrates: indeed their K_m values are in the millimolar range (≈11 mM). NAD^+ is the preferred coenzyme for the low K_m isoenzymes (ALDH1 and ALDH2), whereas the high K_m isoenzymes (ALDH3 and ALDH4) can use either NAD^+ or $NADP^+$.

In addition to the above mentioned genes, a number of additional genes have been cloned and characterized in humans [20]; the major ones will be briefly listed in the following.

ALDH1B (ALDH5): it is expressed in various tissues including liver, brain, adrenal gland, testis, stomach, and parotid gland. *ALDH1A6* (ALDH6): this isoenzyme is primarily expressed in the salivary gland, stomach, and kidney. The cDNA encodes 512 amino acid residues and shows a 70% sequence homology with ALDH1. *ALDH3B1* (ALDH7): the isoenzyme is expressed mainly in the kidneys and lungs; the cDNA encodes 468 residues with a 52% positional identity with ALDH3. *ALDH3B2* (ALDH8): the gene product shows 85% homology with of ALDH7. *ALDH9A1* (ALDH9): the isoenzyme has a high activity for oxidation of gamma-aminobutyraldehyde and other amino aldehydes [8].

Genetic polymorphisms have been reported in a number of *ALDH* genes: nucleotide changes and effect on encoded proteins are listed in *table 2*. Due to the major role of mitochondrial ALDH2 in acetaldehyde oxidation [21], the genetic factor that most strongly correlates with reduced ethanol consumption and incidence of alcoholism is human *ALDH2* functional gene polymorphism. The enzyme is encoded by two distinct alleles in chromosome 6: *ALDH2*1* (wild type allele) and *ALDH2*2*, differing for the substitution glutamate-to-lysine at position 487 (E487K) due to a single point mutation (transition G⇒A). Although the difference between the two alleles appears to be minimal, the proximity in the primary structure between the mutation site and the region containing cysteine residues, very likely involved in the catalytic cycle, is compatible with the phenotypic decrease in ALDH2 activity, associated with the variant genotype. Indeed, individuals homozygous for the mutated *ALDH2*2* allele are completely lacking ALDH2 activity, whereas heterozygous individuals showing the *ALDH2*1,2* genotype maintain about 30–50% of the ALDH activity, shown by individuals carrying wild type gene. Blood acetaldehyde levels of *ALDH2*2* homozygous individuals are 6-to-20 fold higher than in *ALDH2*1* gene carriers, in which acetaldehyde is hardly detectable after ethanol consumption. The acetaldehyde blood concentrations reached in individuals homozygous for *ALDH2*2* cause unpleasant side-effects (flush syndrome) which protects them from alcoholism. However, heterozygous individuals may become heavy drinkers or even alcoholics, thus experiencing the toxic effects due to acetaldehyde production [22].

Orientals show the presence of the inactive ALDH2 isoenzyme phenotype in approximately 50% of the individuals [17, 23], whereas no ALDH2-deficient Caucasian or Negroid have been identified so far [24]. This is the reason why Orientals exhibit intense facial flushing after a mild dose of alcohol as compared to Caucasians, thus affecting their drinking habits. The percentage of heavy and moderate drinkers is higher among Caucasians, while abstainers and infrequent drinkers are more frequent among the Chinese and Japanese [8].

About 40% of the South American Native Indians (Mapuche, Atacamen's, Shuara tribes) showed the presence of *ALDH2*2*, which on the contrary has been detected only in a very small percentage of the North American Indians (Sioux, Navajo and Mestizo) [25].

Microsomal CYP2E1

After ingestion of low amount of alcohol, about 10% of ethanol is metabolized in the liver by the microsomal cytochrome P450 CYP2E1, which catalyzes its oxidation to acetaldehyde and then to acetate [26]. During the reaction, CYP2E1 generates reactive oxygen species (ROS) such as H_2O_2, superoxide anion (O_2^-), hydroxyl (•OH) and substrate-derived radicals (1-hydroxyethyl radical), which can cause oxidative stress, triggering lipid peroxidation, protein inactivation, increased cytokine production, mitochondria and DNA damage leading to cell death [27].

Liver damage associated to ethanol consumption is hypothesized to be due at least partially to oxidative stress associated to its metabolism. Indeed alcohol-induced liver disease (ALD) has been related to the increased production of free radicals as well as to the decreased availability of antioxidants and/or impaired activity of a number of enzymatic systems able to detoxify ROS and their by-products, including Glutathione S-Transferases (GST), superoxide dismutase, glutathione peroxidase and catalase.

The potential sources of ROS in ALD are compartmentalized to (i) microsomes, *via* CYP2E1 and cytochrome P450 reductase; (ii) mitochondria, *via* the electron transport chain; (iii) peroxisomes, *via* fatty acid oxidases; and (iv) cytosol, *via* xanthine oxidase and aldehyde oxidase. However, among all the potential hepatic sources of ROS, CYP2E1 has been a center of attention for its pathogenic role in ALD [28].

In addition to ROS, the 1-hydroxyethyl radicals produced by CYP2E1 during ethanol oxidation bind covalently to proteins forming adducts able to induce autoantibodies which have been found in human alcoholics [29, 30]. These antibodies may represent markers of the production of ethanol-derived free radical ad-

Table 3 | *Polymorphisms of CYP2E1, glutathione S-transferase M1, P1 and T1 genes*

Protein	Gene	Nucleotide change	Effect	RFLP	Chromosomal location
CYP2E1.1	CYP2E1*1A	None (Wild Type)		PstI-/RsaI + (c1 allele)	10q24.3-qter
	CYP2E1*1B	9893C >G		TaqI -	
	CYP2E1*1C	6 repeats in the 5' flanking region			
	CYP2E1*1D	8 repeats in the 5' flanking region		DraI- + XbaI	
CYP2E1.2	CYP2E1*2	1132G > A	His76		
CYP2E1.3	CYP2E1*3	10023G > A	Ile389		
CYP2E1.4	CYP2E1*4	4768G > A	Ile179		
CYP2E1.1	CYP2E1*5A	-1293G > C; -1053C > T; 7632T >A		PstI+/RsaI-/DraI-	
	CYP2E1*5B	-1293G > C; -1053C > T		PstI+/RsaI-(c2 allele)	
	CYP2E1*6	7632T >A		DraI-	
	CYP2E1*7A	261T > A			
	CYP2E1*7B	-71G > T; 261T > A			
	CYP2E1*7C	-261T > A; 280G > A			
GSTM1	GSTM1*A	G519	Lys173		1p13.3
	GSTM1*B	C519	Asn173		
	GSTM1*0	deleted	no expression		
	GSTM1*A/B x2	duplicated	overexpression		
GSTP1	GSTP1*A	A313; C341	Ile105; Ala114		11q13.3
	GSTP1*B	313 A > G	Val105; Ala114		
	GSTP1*C	313 A > G; 341C > T	Val105; Val114		
	GSTP1*D	341C > T	Ile105; Val114		
GSTT1	GSTT1*A	wild-type	wild-type		22q11.23
	GSTT1*0	deleted	no expression		

CYP2E1: cytochrome P450 Isoform CYP2E1; GSTM1: glutathione S-Transferase M1;GSTP1: glutathione S-transferase P1; GSTT1:glutathione S-transferase T1.
**Adapted from [7] and [36] .*

ducts and contribute to the hepatotoxicity of ethanol in promoting immune mechanisms of liver injury [30].

The CYP2E1 protein is regulated both transcriptionally and post-transcriptionally through a substrate-induced protein stabilization; chronic ethanol consumption leads to an increase in CYP2E1 protein, by decreasing its degradation, without affecting its mRNA. Beside ethanol consumption other xenobiotics (acetone), a fatty diet, diabetes, obesity or starvation may lead to CYP2E1 induction, contributing to modulate ethanol metabolism.

The induction of CYP2E1 hepatic content, beside increasing ethanol clearance, has been demonstrated to positively correlate with the generation of hydroxyethyl radicals and lipid peroxidation. Consistently, induction of CYP2E1 has been shown to result in enhanced hepatic injury, whereas inhibition of CYP2E1 was associated with an improvement of these lesions [22]. In addition, increased ethanol metabolism may contribute to the development of alcohol dependence: faster ethanol inactivation during long-term alcohol drinking may increase motivation to consume more alcohol in order to maintain a desired level of ethanol at target sites [31].

The *CYP2E1* gene has been localized to chromosome 10 and consists of 9 exons and 8 introns, encoding a 493-amino acid protein. Ten polymorphic loci on human *CYP2E1* gene have been reported so far, most of them in the promoter and intron regions. In addition, a tandem repeat was identified in *CYP2E1* regulatory region [32]. *CYP2E1* gene polymorphisms are listed in *Table 3*.

A RsaI restriction fragment length polymorphism (RFLP) has been reported in the 5'-flanking region of the *CYP2E1* gene. The rare mutant allele (*c2* allele) lacking the RsaI restriction site has been found to be associated with higher transcriptional activity, protein levels and enzyme activity than the wild-type *c1* allele. Moreover, the frequency of RsaI *c2* allele varies in different populations: the highest frequency has been observed in the Taiwanese (28%) and Japanese populations (19-27%), while the frequency is much lower (1-5%) in Africans [8]. The enhanced transcriptional activity of *CYP2E1 c2/c2* might play a role in the development of severe ALD [33]. In individuals carrying the *ADH3*2* allele, the presence of the *CYP2E1 c2* allele increases the risk of ALD, presumably reflecting increased metabolism of ethanol by CYP2E1. The relevance of combination of different genotypes in modulating the risk is suggested by the fact that in the absence of the *CYP2E1*, *ADH3* genotype itself does not influence the risk of ALD [34].

The polymorphic *CYP2E1*D* has been associated with greater CYP2E1 inducibility and it has been suggested to contribute to the development of alcohol and nicotine dependence. *CYP2E1*1D* allele contains a repeat sequence in the 5' flanking region of the gene that may disrupt negative regulatory elements. Homo- and heterozygous individuals for *CYP2E1*1D* gene were found to have greater CYP2E1 activity after ethanol consumption [32].

CYP2E1 is also involved in the metabolism of various other xenobiotics, including procarcinogens, industrial and environmentally relevant small molecular weight volatile organic chemicals. Therefore, chronic ethanol consumption, leading to CYP2E1 induction, may result in the increased conversion of known hepatotoxic agents to their toxic metabolites [22], possibly explaining the enhanced susceptibility of alcoholics to the adverse effects of industrial solvents [33].

The finding of CYP2E1-mediated bioactivation of xenobiotic in prenatal human brain tissue seems of extreme interest. Significant levels of activity and specific mRNA were detectable between gestational days 45 and 53, a period during which embryogenesis overlaps with organogenesis, taking place at 50-60th days of gestation. The mRNA levels increase up to days 80-84, then remain almost constant throughout the early foetal period [35] and may be increased by ethanol itself or by its strong effect on maternal nutritional status (*i.e.* altered fat or vitamin A and B intake). The presence of CYP2E1 during organogenesis in the brain, the target organ of alcohol teratogenesis, has been associated with the appearance of foetal alcohol syndrome (FAS), as a result of alcohol consumption during pregnancy. FAS is characterised by a particular pattern of facial anomalies, growth retardation and developmental abnormalities in the central nervous system that could result in mental retardation. Even if FAS is not evident, some evidences indicate that adults, who had been prenatally exposed to alcohol, frequently suffer from mental disorders and maladaptive behaviours and are prone to become alcoholics themselves. Damages in the foetal brain due to alcohol consumption by the mother during gestation has been associated to the presence of many polyunsaturated fatty acid side chains in the membranes of embryonic and foetal brain, making the tissue a highly susceptible target for ROS and radicals arising from CYP2E1-mediated ethanol metabolism *in situ*. The damages in the brain caused by lipid peroxidative processes triggered by ROS might be manifested as the central nervous system dysfunction after birth, described as FAS, although other factors such as decreased levels of retinoic acid may act concurrently.

Glutathione S-transferases

Glutathione S-transferases (GST) are phase II xenobiotic metabolizing enzymes, acting as a highly efficient detoxification system. They catalyze the conjugation of harmful electrophilic compounds with reduced glutathione (a tripeptide present at relatively high concentrations in the cytosol), to produce less toxic or readily excreted metabolites. Moreover, these enzymes have a strong antioxidant function and protect cells from the natural by-products of lipid peroxidation and oxidative stress [36].

Since the implication of ROS, generated during ethanol metabolism and by ethanol-induced cell damage, has been postulated in the etiology of alcohol-induced pathologies, GST activity may play a central role by detoxifying both ROS and other ethanol-derived free radicals, as suggested by the alteration of GST expression in the liver of patients with ALD [37].

The cytosolic GSTs are dimeric proteins with each subunit having 22-28 kD MW. On the basis of their amino acid sequence, in humans 8 families of the cytosolic forms have been identified and named with greek symbols, each class consisting of various isoenzymes; GSTs mainly involved in ROS detoxication belong to Alpha, Mu, Theta and Pi families [38].

The α-GST are very abundant hepatic homo- or heterodimers, in humans accounting for about 85% of the total GST protein. The dimerization of two distinct subunits (A1 and A2) gives rise to GSTA1-1, GSTA1-2 and GSTA2-2. Other α-GST have been localized in extrahepatic tissues, such as GSTA3 and GSTA5, mainly expressed in the skin.

Alpha, Mu and Pi GST can detoxify harmful α,β-unsaturated carbonyl, such as 4-hydroxynonenal generated by lipid peroxidation and the products of oxidative DNA damage mediated by free radicals. The alpha-GST, as well as the microsomal membrane bound GST, exhibits glutathione peroxidase activity, suggesting an additional defense mechanism against lipid peroxidation associated with ethanol consumption.

In humans, genetic polymorphisms have been described in *GSTM1*, *GSTT1* and *GSTP1* genes. Among the described polymorphisms at the *GSTM1* locus on chromosome 1p13.3, the most studied encodes for a gene deletion (*GSTM1* null genotype), resulting in a complete absence of GSTM1 enzyme activity. The frequency of the *GSTM1* null genotype ranges from 23 to 62% in different populations and is approximately 50% in Caucasians [38].

For *GSTT1* locus, located on chromosome 12q11.2, one polymorphism has been described. The *GSTT1* null genotype represents a gene deletion and is associated with the absence of functional enzyme activity. The frequency of these null genotypes ranges from 16 to 64% in different populations being approximately 20% in Caucasians [39].

Although the absence of an active GST isoform may be of relevance for the total detoxifying capacity of the cell, compensation mechanisms due to the overlapping substrate specificities exhibited by different GST can limit the consequent functional impairment. However, individuals with the homozygous *GSTM1* or *GSTT1* null genotypes express no protein of two major human GST isoforms, highly express in the gastric and intestinal mucosa, are expected to have a reduced ability to detoxify reactive metabolites resulting from ethanol metabolism.

At least four different polymorphisms have been described at the *GSTP1* locus on chromosome 11, the most important of which encoding for an enzyme with altered activity. Polymorphisms of the *GSTP1* gene consists of an A-to-G transition of nucleotide 313 in exon 5 *(GSTP1*B)* and a C-to-T transition of nucleotide 341 in exon 6 *(GSTP1*C)*, involving the substitution of two amino acids in the enzyme active site, Ile ⇒Val and Ala ⇒ Val. These allelic variants appear to influence GSTP1 activity, therefore modulating the risks of damage [36]. The amino acid 105 is proximal to the enzymatic active site, therefore it is not surprising that this residue can modulate the catalytic activity. The same transition may occur also in position 114, but the functional consequences of this mutation have not been clarified yet.

The GSTP1 Val^{105}/Val^{105} polymorphism is very common and may result either in reduction or increase of the enzyme activity of the compared to the wild type form (Ile^{105}), dependent on the electrophilic structure of the substrate [38]. As an example GSTP1 Val^{105}/Val^{105} genotype has been shown to be protective against asthma symptoms, since the mutated gene is more efficient in scavenging ROS formed during the chronic inflammation process associated with the pathology, thus protecting lung cells from damages produced by oxidative stress [38].

CONCLUSIONS

Ethanol-induced adverse effects result from a broad range of complex interactions between environmental, behavioral, genetic and social factors. There is a high ethnic and interindividual variability in the occurrence and gravity of alcohol related pathologies, often not correlated to the amount of ethanol intake.

In the last years the gender-related different susceptibility has focused the attention on women vulnerability to ethanol and particularly to the possible teratogenic effects, as a consequence of alcohol consumption during gestation, resulting in neurobehavioral disorders in the adult. These differences have been mainly ascribed to ethanol toxicokinetics and polymorphisms of metabolic enzymes, in combination to socio-cultural factors, whose contribution cannot be disregarded.

However, in order to identify the determinants of multifactorial diseases such as ALD and other alcohol-related disorders, evaluation of functional polymorphism at multiple genes is necessary. The identification of possible biomarkers of susceptibility will represent the main goal of the near future and will contribute to the implementation of adequate prevention strategies, to the development of effective diagnostic test strategies, to detect higher risk drinking behavior and early indicators of tissue damage.

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