

FEATURE REVIEW

Genetic polymorphism in ethanol metabolism: acetaldehyde contribution to alcohol abuse and alcoholism

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Acetaldehyde, the first product of ethanol metabolism, has been speculated to be involved in many pharmacological and behavioral effects of ethanol. In particular, acetaldehyde has been suggested to contribute to alcohol abuse and alcoholism. In the present paper, we review current data on the role of acetaldehyde and ethanol metabolism in alcohol consumption and abuse. Ethanol metabolism involves several enzymes. Whereas alcohol dehydrogenase metabolizes the bulk of ethanol within the liver, other enzymes, such as cytochrome *P4502E1* and catalase, also contribute to the production of acetaldehyde from ethanol oxidation. In turn, acetaldehyde is metabolized by the enzyme aldehyde dehydrogenase. In animal studies, acetaldehyde is mainly reinforcing particularly when injected directly into the brain. In humans, genetic polymorphisms of the enzymes alcohol dehydrogenase and aldehyde dehydrogenase are also associated with alcohol drinking habits and the incidence of alcohol abuse. From these human genetic studies, it has been concluded that blood acetaldehyde accumulation induces unpleasant effects that prevent further alcohol drinking. It is therefore speculated that acetaldehyde exerts opposite hedonic effects depending on the localization of its accumulation. In the periphery, acetaldehyde is primarily aversive, whereas brain acetaldehyde is mainly reinforcing. However, the peripheral effects of acetaldehyde might also be dependent upon its peak blood concentrations and its rate of accumulation, with a narrow range of blood acetaldehyde concentrations being reinforcing.

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Introduction

Although the neurobehavioral bases of alcohol consumption have been extensively studied in the past decades, some of the primary mechanisms of ethanol are still unclear. Whereas a number of ethanol's molecular targets have been identified within the brain,¹ basic issues about its mechanisms of action remain unresolved. Among the most controversial issues, the role of acetaldehyde in alcohol abuse and alcoholism remains a matter of intense debate. Acetaldehyde, the first product of ethanol metabolism, is a biologically active molecule. Aside from its well described hepatotoxic effects,² acetaldehyde also induces a range of behavioral effects whose neurochemical basis are still mostly unknown.³ Consequently, acetaldehyde has been speculated to contribute to and even to mediate ethanol's pharmacological and behavioral effects.^{4,5} However, such a hypothesis does not reach a large agreement among scientists in the field of alcohol research. Whereas

some of them deny any role for acetaldehyde in ethanol's effects, others have suggested that alcoholism is in fact a syndrome of 'acetaldehydism'.^{5–7} Considering the possible key role of acetaldehyde in alcohol addiction, it is of critical importance to clarify the respective functions of acetaldehyde and ethanol molecules in the pharmacological and behavioral effects of alcohol consumption. In the present review, we examine recent animal and human studies reporting evidence that acetaldehyde is involved in the pharmacological and behavioral effects of ethanol. The role of acetaldehyde in alcohol abuse and alcoholism will be critically considered with a particular focus on ethanol metabolism. Indeed, pharmacologically relevant acetaldehyde concentrations are required within the brain before any behavioral effect can be attributed to acetaldehyde. To date, it is still unclear whether such a basic requirement is fully satisfied.

Ethanol metabolism: an update

In humans, more than 90% of ingested alcohol is eliminated via metabolic degradation mainly in the liver. Ethanol is first metabolized into acetaldehyde through several enzymatic and nonenzymatic

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mechanisms,⁸ the main enzymatic pathways being alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1) and catalase. Acetaldehyde is subsequently oxidized into acetate through several enzymatic pathways,⁸ although aldehyde dehydrogenase (ALDH) metabolizes the bulk of acetaldehyde in the liver. There is a high individual variability in ethanol metabolism, with alcohol elimination rates varying as much as three- to four-fold from person to person.⁹ Such an individual variability is mainly due to genetic variations in the main ethanol and acetaldehyde metabolizing enzymes. Particularly, there are multiple molecular forms of the enzymes ADH and ALDH. These genetic polymorphisms determine the level of acetaldehyde accumulation after alcohol consumption and therefore have an impact on individual susceptibilities to both alcohol toxic effects and alcoholism.¹⁰

Alcohol dehydrogenase

ADH is a cytosolic enzyme using NAD⁺ as a cofactor. There are multiple molecular forms of ADH in humans. Human ADHs are categorized in five classes on the basis of their structural and kinetic characteristics. However, under physiological conditions, the main isoforms involved in human ethanol metabolism are ADHs from classes I, II and IV.

Class I ADH consists of three isozymes that are widely distributed in the body, although 90% are found in the liver.¹¹ With a low K_m (<5 mM) for ethanol, these enzymes play a major role in ethanol metabolism. There are several polymorphic variants of ADH1, which differ in their efficiency for ethanol oxidation. In the present review, we have adopted the recently recommended new nomenclature for vertebrate ADH.¹² However, since many studies keep using the old nomenclature, Table 1 shows the former names of the main variant alleles for class I ADH along with their translations in the new nomenclature. Two of the three ADH1 enzymes show genetic polymorphism: there are three different alleles for ADH1B and two for ADH1C (Table 1). The enzymes encoded by *ADH1B*2*, *ADH1B*3* and *ADH1C*1* alleles have a greater enzymatic activity, suggesting

a faster ethanol oxidation into acetaldehyde.¹³ Class II ADH has an intermediate K_m for ethanol and plays a less important role in liver ethanol metabolism than ADH1. ADH2 is almost exclusively expressed in the liver.⁸ Although widely distributed in the body, ADH3 plays only a very minor role in hepatic ethanol metabolism. Indeed, its very high K_m for ethanol results in poor ethanol oxidation properties.¹⁴ However, recent studies have suggested that ADH3 contributes to the first-pass ethanol metabolism in the stomach, where very high ethanol concentrations may be found after alcohol drinking.¹⁵ ADH4 is primarily located in the stomach mucosa, but is also found in epithelial tissues of the upper digestive tract.¹⁶ ADH4 displays poor ethanol catalytic properties. However, this ADH might also be involved in the first-pass metabolism of ethanol in the stomach.¹⁷ An additional ADH5 has recently been discovered, but its tissue distribution and properties remain to be determined.¹⁸

Microsomal ethanol oxidizing system

Although ADH accounts for the greater part of ethanol oxidation, a microsomal ethanol oxidizing system involving mostly the CYP2E1 has also been identified.¹⁹ In addition to CYP2E1, two other cytochromes, CYP1A2 and CYP3A4, also contribute to the microsomal ethanol oxidizing system, although to a lesser extent.^{20,21} The microsomal ethanol oxidizing system accounts for the major non-ADH ethanol metabolism in the liver.²² However, CYP2E1 has a low ethanol catalytic efficiency relative to ADH and therefore is responsible for only a small part of total ethanol metabolism. Nevertheless, CYP2E1 displays a singular characteristic in ethanol metabolism, that is, its inducibility.²³ Indeed, previous studies have shown that the activity of CYP2E1 is increased up to 10-fold after chronic alcohol consumption.^{19,24–26} Therefore, while the largest part of ingested ethanol is normally metabolized by ADH, CYP2E1 is significantly involved in ethanol metabolism at high ethanol concentrations or after long-term ethanol intake. After chronic alcohol consumption, CYP2E1 increases the rate of ethanol clearance and is therefore accountable for the metabolic tolerance to ethanol.^{19,22,24–26} CYP2E1 induction after chronic ethanol consumption also leads to higher acetaldehyde concentrations. Such an effect is further aggravated by the decreased activity of ALDH after chronic ethanol intake.²⁷ Genetic polymorphisms in CYP2E1 genes have been described.^{28–30} In particular, a rare mutant allele, named the *c2* allele (*CYP2E1*5B*), is associated with higher transcriptional activity and protein levels and an elevated enzyme activity relative to the common wild-type *c1* allele (*CYP2E1*5A*).³¹

Catalase

The enzyme catalase has also been shown to oxidize ethanol into acetaldehyde within the peroxisomes.³² This process is hydrogen peroxide (H₂O₂) dependent. However, under normal physiological conditions,

Table 1 New and old nomenclatures for human class I ADH variants^a

Protein	Allele	Former allele nomenclature	Particular properties
ADH1A	<i>ADH1A</i>	<i>ADH1</i>	
ADH1B1	<i>ADH1B*1</i>	<i>ADH2*1</i>	
ADH1B2	<i>ADH1B*2</i>	<i>ADH2*2</i>	Faster ethanol oxidation
ADH1B3	<i>ADH1B*3</i>	<i>ADH2*3</i>	Faster ethanol oxidation
ADH1C1	<i>ADH1C*1</i>	<i>ADH3*1</i>	Faster ethanol oxidation
ADH1C2	<i>ADH1C*2</i>	<i>ADH3*2</i>	

^aBased on Duester *et al.*¹⁰

catalase plays only a minor role in ethanol metabolism,²² but its contribution might be enhanced in the presence of higher amounts of H₂O₂.³³ Furthermore, catalase may be an alternative metabolic pathway for ethanol oxidation within the brain, where ADH and CYP2E1 appear to be of minor importance for ethanol metabolism.^{34,35} However, the precise role of catalase in ethanol elimination and especially in brain ethanol metabolism remains a matter of debate.

Nonoxidative ethanol metabolism

In addition to the main oxidative pathways, ethanol is also metabolized, although to a minor extent, by a nonoxidative pathway to form fatty acid ethyl esters.²³ This reaction is catalyzed by fatty acid ethyl ester synthases and may be of importance in organs lacking ethanol oxidative metabolism, such as the heart.³⁶ Fatty acid ethyl esters resulting from this nonoxidative ethanol metabolic reaction are involved in alcohol-induced organ injuries.³⁶

Aldehyde dehydrogenase

The second step of ethanol metabolism is mainly mediated by ALDH. This enzyme rapidly converts acetaldehyde to acetate. Like ADH, ALDH enzymes use NAD⁺ as a cofactor in the oxidation of acetaldehyde. In humans, there are multiple forms of ALDH that are divided into nine major families.³⁷ However, only some of these isoforms are significantly involved in acetaldehyde metabolism, while the others metabolize a variety of substrates (Table 2). To date, a significant role in acetaldehyde oxidation has been identified only for ALDH1A1, ALDH1B1 and ALDH2.³⁸ However, the mitochondrial ALDH2 plays the central role in human acetaldehyde metabolism because of its very low K_m (< 5 μ M) for acetaldehyde. ALDH2 is highly expressed in the liver and stomach, but is also widely distributed in other tissues

including the brain.³⁹ As for ADH, there is an important genetic polymorphism in human ALDH.³⁸ The allele *ALDH2*2* has been particularly studied because it causes a catalytic inactivation of the enzyme and alters acetaldehyde metabolism.^{40,41} This deficient allele has a relatively high frequency in Asians, but is also found in other populations.⁴²

Brain acetaldehyde accumulation

A prerequisite for the involvement of acetaldehyde in ethanol behavioral effects is the occurrence of pharmacologically significant levels of acetaldehyde in the brain after alcohol consumption. Brain acetaldehyde levels have been an extremely controversial topic for the last two decades. Several studies have reported that peripherally produced acetaldehyde penetrates into the brain with difficulty due to the presence of ALDH in the microvasculature of the brain.⁴³ To overcome this metabolic barrier, very high blood acetaldehyde concentrations (> 100 μ M) are necessary.³⁵ Since blood acetaldehyde concentrations are generally very low after oral alcohol consumption,^{44,45} it has been concluded that peripheral acetaldehyde cannot reach the brain under normal conditions. As it was initially believed that the brain lacks any physiologically relevant alcohol metabolizing system,⁴⁶ earlier studies concluded that acetaldehyde cannot play a significant role in ethanol behavioral and neurochemical effects. Indeed, ADH, the main ethanol oxidizing pathway, is not physiologically active in the brain.³⁵ Only ADH3 is generally found in human brain⁴⁷ and its very high K_m for ethanol makes that enzyme virtually useless for ethanol metabolism even after severe alcohol intoxication. However, evidence of brain ethanol oxidizing properties has slowly emerged with the early observation that ethanol interacts with brain catalase *in vivo*.⁴⁸ Further studies have demonstrated that both

Table 2 Human ALDH polymorphism^a

Gene	Major substrate	Acetaldehyde catalytic efficiency (when relevant)
<i>ALDH1A1</i>	Retinal	Moderate (K_m 50–100 μ M)
<i>ALDH1A6</i>	Aliphatic aldehyde, retinal	
<i>ALDH1A7</i>	Retinal	
<i>ALDH1B1</i>	Propionaldehyde	High (K_m 30 μ M)
<i>ALDH1L1</i>	Folate	
<i>ALDH2</i>	Acetaldehyde	Very high (K_m < 5 μ M)
<i>ALDH3A1</i>	Fatty and aromatic aldehyde	
<i>ALDH3A2</i>	Fatty and aromatic aldehyde	
<i>ALDH3B1</i>	Aliphatic and aromatic aldehyde	
<i>ALDH3B2</i>	Undefined	
<i>ALDH4A1</i>	Glutamate γ -semialdehyde	
<i>ALDH5A1</i>	Succinic semialdehyde	
<i>ALDH6A1</i>	Methylmalonate semialdehyde	
<i>ALDH7A1</i>	Undefined	
<i>ALDH8A1</i>	Undefined	
<i>ALDH9A1</i>	Amine aldehyde	

^aBased on Vasiliou *et al.*²⁷

astrocytes in culture and rat brain homogenates are able to produce biologically significant concentrations of acetaldehyde from perfused ethanol.^{34,49–51} These studies have also shown that catalase plays a critical role in this process, as the addition of various catalase inhibitors to brain homogenates reduces acetaldehyde accumulation after ethanol perfusion.^{34,50} However, other pathways might also be involved in ethanol metabolism and acetaldehyde production within the brain. A recent study has reported the presence of ADH1 and ADH4 within very specific rat brain regions such as the cerebellum, hippocampus and cerebral cortex, suggesting that these enzymes might contribute to acetaldehyde production in these brain areas.⁵² However, it is still unknown whether these enzymes contribute to ethanol metabolism in human brain. CYP2E1 might also contribute to ethanol metabolism in the brain, although its actual role is still unclear.³⁵ The specific ethanol oxidizing cytochrome, CYP2E1, is expressed in various rat brain regions.⁵³ In addition, several studies have revealed that brain CYP2E1 is also inducible by chronic ethanol treatments.^{54–56} Since the activity of ALDH is significantly reduced by chronic ethanol,²⁷ brain acetaldehyde accumulation might be enhanced after chronic alcohol consumption. Therefore, acetaldehyde might play a more important role in mediating the neuropharmacological and behavioral effects of ethanol in chronic alcohol abusers. Finally, there is evidence that an additional still unidentified pathway contributes to brain acetaldehyde production.^{51,57} In summary, the brain is able to produce biologically significant acetaldehyde levels from ethanol metabolism. While there is evidence that catalase is involved in this process, other pathways that still require clarification might also play a significant role in brain ethanol metabolism.

Rewarding properties of acetaldehyde in animal studies

It is generally assumed that the primary factor that controls the propensity to consume ethanol is its positive reinforcing properties.⁵⁸ In the last decades, a number of authors have suggested that acetaldehyde contributes to or even mediates the reinforcing effects of ethanol. In animal studies, three lines of evidence argue in favor of this hypothesis. First, there is evidence that acetaldehyde is a molecule with strong rewarding properties in animals. Other studies have reported an association between ethanol preference and brain acetaldehyde production in various rodent strains and lines. Finally, alterations of ethanol metabolism have been shown to modulate ethanol preference and consumption.

The first evidence that acetaldehyde might be involved in the reinforcing effects of ethanol come from studies demonstrating that acetaldehyde is highly reinforcing in rats.⁵⁹ Earlier studies have shown that acetaldehyde is readily self-administered

both intracerebroventricularly^{60,61} and intravenously.^{62,63} In addition, these studies have reported that acetaldehyde self-administration is easier to establish in rats than ethanol self-administration,⁶¹ indicating that acetaldehyde is more reinforcing than its parent drug, ethanol. This conclusion has recently been supported by an intracranial self-administration experiment.⁶⁴ This study has shown that acetaldehyde is a 1000-fold more potent reinforcer than ethanol when tested for self-administration into the ventral tegmental area, a brain region strongly involved in ethanol reinforcing effects. In addition, several studies have reported that acetaldehyde self-administration in rats correlates with subsequent voluntary ethanol consumption,^{62,63} suggesting that acetaldehyde is involved in the regulation of ethanol intake. Place conditioning studies have further demonstrated that acetaldehyde is primarily reinforcing in rats. These studies have shown that rats develop a conditioned preference for a place in which they had previously experienced acetaldehyde administration via either the intracerebroventricular or the intraperitoneal route.^{65,66} In contrast, ethanol-induced place preferences are quite difficult to obtain in rats, as they generally display a place aversion after conditioning with ethanol.^{65,67–69} Therefore, place conditioning studies further support the notion that acetaldehyde is more reinforcing than ethanol in rats.

Indirect evidence of the reinforcing properties of acetaldehyde come from studies that have investigated the relationships between ethanol metabolism and ethanol consumption in rodents. In particular, the activity of brain catalase significantly and positively correlates with voluntary ethanol consumption in rats,^{70,71} although conflicting results have been obtained in mice.⁷² Rats with higher brain catalase activity generally consume more ethanol. Since catalase is involved in acetaldehyde production within the brain, these studies have concluded that acetaldehyde significantly contributes to the reinforcing effects of ethanol. With a reduced catalase activity, less acetaldehyde is produced within the brain, ethanol is therefore less reinforcing and as a consequence these rodents consume less alcohol. Several studies have also investigated the effects of pharmacological modulations of ethanol metabolism on the propensity to consume alcohol. Overall, these studies are coherent with the notion that brain acetaldehyde is involved in the reinforcing effects of ethanol. Pharmacological inhibition of catalase has been shown to reduce ethanol consumption,^{73,74} in agreement with the idea that the reinforcing properties of brain acetaldehyde contribute to the motivation to drink alcohol. However, the enzyme catalase is also involved in a number of other cellular functions, such that it remains possible that the effects on alcohol consumption are explained by other functions of catalase than the production of acetaldehyde from ethanol.^{75,76}

Although the above-mentioned studies consistently support a role for acetaldehyde in the reinforcing

properties of ethanol, other results apparently argue against this theory. For example, negative correlations have been observed between blood acetaldehyde concentrations and alcohol preference.⁷⁷ Rats and mice with lower hepatic ALDH activity, resulting in blood acetaldehyde accumulation, avoid drinking alcohol.^{78,79} Finally, pharmacological inhibition of ALDH suppresses alcohol drinking in rats⁸⁰ in the same way as disulfiram prevents alcohol consumption in humans. The reasons for such discrepancies between animal studies are not known with certainty. However, it can be hypothesized that acetaldehyde induces opposite hedonic effects depending on the localization of its accumulation. In the brain, acetaldehyde induces strong reinforcing effects as demonstrated by a number of studies.^{60,61,64,66} In contrast, its peripheral accumulation produces adverse effects that prevent further alcohol consumption. In each experimental condition, the overall hedonic contribution of acetaldehyde will be dependent on its relative peripheral and brain concentrations. In some cases, the brain reinforcing effects of acetaldehyde may overshadow its peripheral adverse effects. This is believed to happen when acetaldehyde is directly injected in rodents via the intraperitoneal or intravenous route.⁸¹ In other circumstances, for example when the enzyme ALDH is inactive,⁷⁸ the peripheral aversive effects of acetaldehyde would predominate and therefore reduce the propensity to drink alcohol.

Genetic polymorphism of ethanol metabolizing enzymes in humans

Although many genes are believed to contribute to alcoholism susceptibility,⁸² studies on the genetic polymorphism in alcohol-metabolizing enzymes have provided the most consistent results. In these studies, genetic polymorphism in ADH and ALDH has been shown to be associated with alcohol drinking habits and the susceptibility to develop alcohol abuse and alcoholism. Although acetaldehyde accumulation is often suggested to mediate these effects, a causal role for acetaldehyde is not clearly established in most cases.

Genetic polymorphism of alcohol dehydrogenase

Genetic variants of both *ADH1B* and *ADH1C* genes have been especially studied for their role in the susceptibility to develop alcoholism.⁸³ *ADH1B2*, *ADH1B3* and *ADH1C1* variants show a faster rate of ethanol oxidation and are therefore believed to promote acetaldehyde accumulation.⁸⁴

Although *ADH1B*1* is the predominant allele in most populations, the *ADH1B*2* allele may be found in frequencies up to 90% in some Asian populations.^{42,85} In European populations, this atypical form of *ADH1B* occurs with a low frequency usually around 5%.^{42,86} In contrast, the *ADH1B*3* allele occurs almost exclusively in Africans, African-Americans and in some Native Americans, and is found only in extremely low frequencies in other popula-

tions.^{87,88} The *ADH1B*2* allele has been shown to reduce the occurrence of alcohol abuse and alcoholism in Asians, in Whites and in Jewish populations in which this allele has a relatively high prevalence.^{89,90} Several studies have reported that *ADH1B* allele frequencies differ between alcoholics and nonalcoholics, with a higher occurrence of the atypical form, *ADH1B*2*, in nonalcoholics^{85,86,91–104} and in moderate drinkers relative to heavy drinkers.^{90,105,106} Similarly, the *ADH1B*3* allele is associated with lower rates of heavy drinking and alcohol dependence in Native Americans,¹⁰⁷ and with a negative family history of alcoholism in African-Americans.¹⁰⁸ In a meta-analysis, Whitfield⁸³ has concluded that the *ADH1B*1* allele is associated with a three-fold increase in the risk of alcoholism relative to the *ADH1B*2* allele. Since the *ADH1B*2* allele codes for an enzyme with a faster ethanol oxidation rate, it is generally assumed that this allele protects against alcohol abuse and alcoholism because of the unpleasant symptoms associated with acetaldehyde accumulation after alcohol drinking.¹⁰⁹ However, additional studies have shown that this allelic variation does not result in an increased blood acetaldehyde concentration after alcohol drinking,^{9,110} despite a higher rate of ethanol elimination.¹¹¹ In agreement with this observation, the frequency of facial flushing, a sign typically associated with high blood acetaldehyde concentrations, is similar in individuals carrying different *ADH1B* alleles,^{106,112} although another study reports an increase in the self-reported flushing in individuals carrying at least one *ADH1B*2* allele.¹¹³ Furthermore, the effects of *ADH1B*2* and *ALDH2*2* alleles on alcoholism susceptibility appear to be independent of each other.^{114,115} Since there are good indications that blood acetaldehyde accumulation is involved in the protection against alcoholism afforded by the *ALDH2*2* allele,^{112,116} this latter observation argues against a role for acetaldehyde accumulation in the effect of *ADH1B* polymorphism on alcoholism vulnerability. Therefore, the mechanisms of *ADH1B*2* protection against alcoholism may not be mediated through the adverse effects of acetaldehyde and remain to be clarified.

The frequency of *ADH1C*1* allele is about 50% in European populations, while it may be found in frequencies up to 90% in some Asian and African populations.^{42,85,87,100,117} This allele is also suggested to provide a protection against alcohol abuse and alcoholism, as demonstrated by a higher frequency of this allele in nonalcoholics, especially from Asian populations.^{92,93,100,102,103,118} However, the effects of *ADH1C*1* allele on alcoholism susceptibility are still a matter of debate. Several studies have shown that the effects of the *ADH1C*1* allele are considerably smaller than those of *ADH1B*2*.^{91,104} In European populations, no association is usually found between *ADH1C* allelic variations and alcoholism incidence.^{86,119} In addition, differences in the frequency of *ADH1C*1* alleles between alcoholics and nonalcoholics from Asian populations might be attributed

only to a strong linkage disequilibrium between the functional variants in *ADH1B* and *ADH1C* genes.^{114,120} When this disequilibrium is controlled, the *ADH1B* allelic variation is sufficient to explain differences in alcoholism incidence and the effects of *ADH1C* on the susceptibility to alcoholism become nonsignificant. Therefore, these recent studies have concluded that *ADH1C* polymorphism has no demonstrated influence on the susceptibility to develop alcoholism, although a recent study reports additional evidence supporting *ADH1C* as a candidate gene that slightly affects the vulnerability to alcoholism.¹²¹

The discovery of new allelic variations in *ADH* genes should help to clarify the role of *ADH1B* and *ADH1C* variants in the protection against alcoholism. Recently, a new *ADH1C* allele has been identified in Native Americans, which results in the substitution of a proline by a threonine in codon 351 and has the potential for altering the functional characteristics of the enzyme.¹²² Additional polymorphisms have also been identified in other Class I *ADH* sites: *ADH1B* *RsaI*, *ADH1C* *HaeIII* and *ADH1C* *EcoRI*.^{87,120} In an American Indian population, both *ADH1C* *HaeIII* and *ADH1C* *EcoRI* sites exhibit linkage to binge drinking, whereas *ADH1C* *HaeIII*-site-present allele is associated with a slight increase in alcohol dependence.¹²¹ However, it remains unclear whether these variants have a direct effect or whether they are in linkage disequilibrium with other markers that affect alcohol consumption and alcoholism. It is also worth noting that these later results have not been replicated in a second sample from the same population, although the reasons for such a difference are unclear.¹²¹ Further studies using haplotype analyses should clarify these questions. In the collaborative study on the genetics of alcoholism (COGA), a protective region against alcoholism has been identified on chromosome 4 where the *ADH* genes reside.^{123–125} However, since most of the subjects in the COGA samples are Caucasians who have a very low frequency of *ADH1B**2 and *ADH1B**3 alleles, these results further suggest that a new protective *ADH* allele or other genes located nearby remain to be identified. These possible protective alleles might be located on the genes encoding other *ADH*s than Class I. For instance, several polymorphisms have been identified in the *ADH2* gene.^{126,127} In particular, a variant allele, –75 bp (A/C), in the proximal promoter of *ADH2* leads to higher expression of the gene.¹²⁶ Since *ADH2* contributes to ethanol metabolism after moderate and high alcohol intake, this allele might alter acetaldehyde concentrations and therefore has been suggested to affect the risk for alcoholism.¹²⁶ However, further studies are needed to substantiate this hypothesis.

Genetic polymorphism of CYP2E1

The effects of CYP2E1 polymorphisms on the susceptibility to develop alcoholism remain controversial. So far, most genetic studies on the association

between CYP2E1 functional polymorphism and alcoholism have yielded negative results.^{30,128} There are no differences in *c2* allele (*CYP2E1**5B) frequencies between alcoholics and nonalcoholics,^{96,115,129,130} and between moderate and heavy drinkers,^{106,131} although two recent studies have reported a greater alcohol consumption¹³² and a higher prevalence of alcoholism¹¹⁸ in individuals carrying the *c2* allele. Therefore, CYP2E1 polymorphism is usually not believed to be related to alcohol abuse and alcoholism,¹²⁸ whereas there is evidence that it is associated with the susceptibility to develop alcohol liver disease.¹³³

Genetic polymorphism of catalase

Although brain catalase activity correlates with voluntary ethanol consumption in rodents,^{70,71} very few studies have investigated the effects of individual variability in the activity of human catalase. In a study with human subjects, Koechling and Amit¹³⁴ have shown that blood catalase activity significantly correlates with alcohol consumption. Since there is also a very significant positive correlation between blood and brain catalase activities in rats,⁷⁰ Koechling and Amit¹³⁴ have concluded that human brain catalase activity modulates the motivation to drink alcohol. This conclusion is further supported by a subsequent study showing that subjects with a familial history of alcoholism have a higher mean catalase activity than control subjects.¹³⁵ However, the genetic basis for this relationship between catalase activity and alcohol consumption remains unknown. So far, other studies on the genetic polymorphism of catalase have found no association with alcohol drinking behaviors,¹³⁶ but this question clearly requires further investigations.

Genetic polymorphism of aldehyde dehydrogenase

Most of *ALDH* genetic polymorphism studies have focused on the effects of the *ALDH2**2 allele on alcohol consumption and alcoholism incidence. Indeed, this allele encodes an inactive form of the mitochondrial *ALDH2*.^{41,137} In particular, *ALDH2**2 homozygosity results in a total loss of *ALDH2* activity. Since *ALDH2* is the main enzyme of acetaldehyde metabolism, individuals carrying the *ALDH2**2 allele show high blood acetaldehyde concentrations after the intake of only moderate alcohol amounts.¹¹⁶ Other *ALDH* genes also show genetic variation,¹⁰ although their involvement in alcohol abuse and alcoholism has not yet been thoroughly studied.

The *ALDH2**2 allele provides a strong protection against alcoholism, especially in *ALDH2**2 homozygous.¹³⁸ A number of studies have reported a reduced frequency of *ALDH2**2 allele in alcoholics relative to non-alcoholics.^{85,92–94,96,97,100,102,139–141} It has been calculated that the *ALDH2**2 allele provides a 10-fold reduction in the risk of alcohol dependence.⁸⁵ Individuals carrying the *ALDH2**2 allele also drink less alcohol^{95,142–145} and have a lower prevalence of binge drinking.^{143–145} Whereas the mutant *ALDH2**2 gene is rarely found in Caucasian and Negroid

populations, it is widely prevalent in some Asian populations, in which it may have a 50% prevalence.⁴² Such a high prevalence of the *ALDH2*2* allele is believed to explain the lower incidence of alcoholism in these populations. In addition to the site that defines the deficiency allele, a number of other variable sites have been identified in the gene that encodes ALDH2.^{128,146–149} Haplotypes studies have shown strong linkage disequilibrium among these variants.^{148,149} However, additional studies are required to determine whether these variants show association with alcoholism and alter acetaldehyde metabolism.

The role of acetaldehyde in the protection against alcoholism provided by the *ALDH2*2* allele is much more convincing than for ADH polymorphism. Indeed, it has been clearly shown that *ALDH2*2* homozygous have considerably higher blood acetaldehyde concentrations after alcohol consumption.^{116,150–152} In addition, a causal link has been established between these higher blood acetaldehyde levels and the adverse response of these individuals to ethanol consumption.¹⁵³ Indeed, in *ALDH2*2* homozygous, alcohol consumption results in a range of unpleasant effects, such as cardiovascular effects, dysphoria, palpitations, dry mouth, headache, nausea and facial flushing, which are attributed to acetaldehyde accumulation.^{116,154–156} Particularly, *ALDH2*2* homozygous have a very high prevalence of facial flushing after alcohol consumption.^{157–158} The experience of these unpleasant symptoms would lead individuals carrying the *ALDH2*2* allele to abstain or moderate further alcohol consumption, thereby reducing their susceptibility to develop alcoholism. It was initially believed that homozygous for *ALDH2*2* are totally protected against alcoholism. However, an alcoholic patient with *ALDH2*2* homozygosity has recently been reported.¹⁵⁹ It is therefore possible to overcome the protection provided by this genotype.¹⁶⁰

Pharmacological manipulations of ethanol metabolism in humans

In humans, manipulations of ethanol metabolism have mostly consisted in the inhibition of ALDH. Indeed, the adverse effects of blood acetaldehyde accumulation have been used as a basis for treating chronic alcoholics with ALDH inhibitors. Such treatment was initially based on the concept of aversion therapy in which ALDH inhibitors are given in order to punish alcohol drinking by an adverse reaction.¹⁶¹ ALDH inhibitors such as disulfiram (Antabuse) and calcium carbimide (Abstem, Temporal) have been used with mixed success to prevent relapse in alcoholics.^{162–164} After alcohol consumption, both drugs lead to high blood acetaldehyde levels that result in a range of unpleasant effects.^{165,166} Although some studies have reported that treatments with ALDH inhibitors effectively reduce alcohol relapse in withdrawn alcoholics,¹⁶² it remains unclear whether their protective effects against alcohol re-

lapse are actually based on the adverse effects induced by acetaldehyde. Instead, the reduction of alcohol consumption in ALDH inhibitors-treated alcoholics might be due to their belief that a harmful and potentially fatal reaction could occur after alcohol drinking. Therefore, it has been suggested that ALDH inhibitors act mainly by creating fear of an alcohol reaction rather than punishing actual alcohol drinking.¹⁶¹ In agreement with the idea that the effects of ALDH inhibitors are more complicated than previously believed, some individuals have been reported not only to keep drinking alcohol despite being treated with disulfiram but even to find the interaction ethanol–disulfiram pleasurable.^{167–170} These latter results suggest that acetaldehyde might also induce rewarding effects in humans, although the conditions for acetaldehyde accumulation to be rewarding are not known.

Conclusion and perspectives

There is an apparent contradiction between the results of animal and human studies with regard to the role of acetaldehyde in alcohol consumption and abuse. Most of animal studies have clearly shown that directly administered acetaldehyde is mainly reinforcing even when peripherally injected. In contrast, human studies have mainly emphasized the aversive consequences of acetaldehyde accumulation. In humans, acetaldehyde accumulation appears to provide a protection against alcohol abuse and alcoholism. In particular, ALDH deficiency and the pharmacological inhibition of ALDH activity have been shown to prevent further alcohol consumption. However, as mentioned above for animal studies, acetaldehyde might exert a dual action according to the localization of its accumulation. While a peripheral accumulation induces a range of unpleasant symptoms that prevent further alcohol drinking, the reinforcing action of brain acetaldehyde is believed to promote alcohol consumption. According to this theory, the overall result of acetaldehyde accumulation would be a balance between its central reinforcing and peripheral aversive effects. In some cases, both effects occur simultaneously with one of them overshadowing the other. For example, it has been speculated that peripheral injections of high acetaldehyde doses to rodents lead to significant brain acetaldehyde levels, whose reinforcing effects would mask the adverse reactions to peripheral acetaldehyde.^{3,65,81} In other circumstances, either the central reinforcing action or the peripheral aversive effects clearly prevail. This is the case when acetaldehyde is directly infused into the brain leading to very strong reinforcing effects.⁶⁴ Therefore, it might be hypothesized that ALDH deficiency and pharmacological inhibition in humans mainly increase peripheral acetaldehyde concentrations with little impact on brain levels. In such conditions, the adverse effects of acetaldehyde should prevail and account for the reduced alcohol consumption. However, this explanation still awaits

experimental confirmations. In particular, there is virtually no data on human brain acetaldehyde concentrations after alcohol consumption in various conditions. Furthermore, very few studies have specifically investigated the effects of brain acetaldehyde on alcohol consumption in humans. The reasons for such a lack of studies are both methodological and ethical. Indeed, it would be unacceptable to administer high doses of such a highly toxic compound as acetaldehyde. Nevertheless, a few studies have indirectly tested the role of brain catalase in alcohol consumption.^{134,135} As described above, the results of these studies support the notion that brain acetaldehyde is reinforcing in humans and therefore promotes alcohol consumption. Clearly, this question requires further studies to clarify the role of brain acetaldehyde in human alcoholism. The effects of acetaldehyde on drinking behaviors might also be strongly dependent on both its peak levels in the blood and its rate of accumulation. It has been suggested that a narrow range of acetaldehyde concentrations is reinforcing and once an upper limit of acetaldehyde concentrations is attained, the aversive effects of acetaldehyde predominate.⁴ Such a hypothesis might explain some intriguing observations in humans. For example, it has been reported that Asian flushers, especially those with *ALDH2**1/2*2 heterozygosity, experience significantly more positive feelings after alcohol intoxication,¹⁷¹ although this allele is associated with a lower prevalence of alcohol abuse and alcoholism. This may also explain the very high alcoholism prevalence among Native Americans,¹⁰⁷ despite a higher incidence of flushing reactions after alcohol drinking in these populations. It is worth noting that the flushing reaction in Native Americans is milder and is not mediated by the *ALDH2**2 allele.¹⁷² Therefore, their levels of acetaldehyde might actually stimulate drinking as opposed to the higher and faster acetaldehyde accumulation that inhibits drinking in Asians with *ALDH2**2 alleles.

In the past decades, it has been speculated that many, if not all of the pharmacological and behavioral effects of ethanol should be attributed to its first metabolite, acetaldehyde. It was even suggested that alcoholism should be renamed 'acetaldehydism'.⁵⁻⁷ Animal studies reported evidence that acetaldehyde is involved in the stimulant, sedative, hypnotic, amnesic, reinforcing, aversive and lethal effects of ethanol.³ However, it is worth noting that the role of acetaldehyde in most of these effects is still questionable and requires further confirmation. In opposition to the radical opinion attributing most of ethanol's effects to acetaldehyde, ethanol and acetaldehyde molecules more likely act synergistically to produce the wide range of effects induced by alcohol consumption. Recent studies also indicate that acetaldehyde is unlikely to be involved in all of the pharmacological effects of ethanol. For example, in drug discrimination studies, acetaldehyde does not substitute for ethanol.^{173,174} In these studies, rats have been trained to discriminate ethanol from water on

the basis of the interoceptive and subjective effects of ethanol. In generalization tests, these rats identify other sedatives, such as pentobarbital and diazepam, as being similar to ethanol. In contrast, acetaldehyde-induced interoceptive cues fail to generalize for those of ethanol. These results demonstrate that acetaldehyde and ethanol generate qualitatively different interoceptive cues, and therefore indicate that acetaldehyde does not mediate all of ethanol's effects.

Finally, the neurochemical basis of acetaldehyde behavioral effects remain to be identified. To date, very few studies have investigated the effects of acetaldehyde on different neurotransmitter systems in relation to changes in behavior. In particular, it should be tested whether ethanol and acetaldehyde share similar neurochemical mechanisms. Since brain acetaldehyde induces robust reinforcing effects, especially when administered directly into the ventral tegmental area,⁶⁴ it can be hypothesized that the mesolimbic dopaminergic pathway is the brain substrates for acetaldehyde reinforcing effects. This pathway is believed to be the major substrate of reward and reinforcement for both natural rewards and addictive drugs.¹⁷⁵ Most of the abused drugs, including ethanol, have been shown to stimulate dopamine release within the nucleus accumbens, one of the targets of ventral tegmental area neurons.¹⁷⁶ Very recently, acetaldehyde itself has been shown to increase the firing rate of dopaminergic neurons from the ventral tegmental area, suggesting that an increased release of dopamine might also mediate acetaldehyde reinforcing effects.¹⁷⁷ In addition, acetaldehyde condenses with dopamine to form salsolinol (6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline), a pharmacologically active compound with strong reinforcing properties.^{178,179} Therefore, this condensation product might also contribute to the overall reinforcing action of acetaldehyde.³ It is clear that further studies are required to unravel the dopaminergic effects of acetaldehyde, as well as its other neurochemical effects.

In conclusion, there is substantial evidence that acetaldehyde at least contributes to the reinforcing effects of ethanol and is therefore involved in alcohol consumption and abuse. However, the role of brain acetaldehyde in humans needs to be clarified in further studies, while its neurochemical basis should be unraveled. Such studies might lead to new therapeutic approaches of alcohol abuse based on alterations in the metabolism of brain acetaldehyde.

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