Polymorphisms in the mitochondrial aldehyde dehydrogenase gene (*Aldh2*) determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats

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Dependence on alcohol, a most widely used drug, has a heritability of 50–60%. Wistar-derived rats selectively bred as low-alcohol consumers for many generations present an allele (*Aldh2*^{2}) of mitochondrial aldehyde dehydrogenase that does not exist in high-alcohol consumers, which mostly carry the *Aldh2*^{1} allele. The enzyme coded by *Aldh2*^{2} has a four- to five-fold lower affinity for NAD^+ than that coded by *Aldh2*^{1}. The present study was designed to determine whether these polymorphisms account for differences in voluntary ethanol intake and to investigate the biological mechanisms involved. Low-drinker F_0 *Aldh2*^{2}/*Aldh2*^{2} rats were crossed with high-drinker F_0 *Aldh2*^{1}/*Aldh2*^{1} rats to obtain an F_1 generation, which was intercrossed to obtain an F_2 generation that segregates the *Aldh2* alleles from other genes that may have been coselected in the breeding for each phenotype. Data show that, with a mixed genetic background, F_2 *Aldh2*^{1}/*Aldh2*^{1} rats voluntarily consume 65% more alcohol ($P<0.01$) than F_2 *Aldh2*^{2}/*Aldh2*^{2} rats. A major phenotypic difference was a five-fold higher ($P<0.0025$) peak blood acetaldehyde level following ethanol administration in the lower drinker F_2 *Aldh2*^{2}/*Aldh2*^{2} compared to in the higher drinker F_2 *Aldh2*^{1}/*Aldh2*^{1} animals, despite the existence of identical steady-state levels of blood acetaldehyde in animals of both genotypes. Polymorphisms in *Aldh2* play an important role in: (i) determining peak blood acetaldehyde levels and (ii) modulating voluntary ethanol consumption. We postulate that the markedly higher peak of blood acetaldehyde generated in *Aldh2*^{2}/*Aldh2*^{2} animals is aversive, leading to a reduced alcohol intake in *Aldh2*^{2}/*Aldh2*^{2} versus that in *Aldh2*^{1}/*Aldh2*^{1} animals. *Pharmacogenetics and Genomics* 15:000–000 © 2005 Lippincott Williams & Wilkins.

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Introduction

A number of studies have shown that alcoholism has a genetic component of 50–60% [1–4]. To unravel the genetic factors involved, research groups in Canada [5], Chile [6], Finland [7], Italy [8] and the USA [9] have selectively bred rats for their low- or high-alcohol consumption. Alcohol-preferring strains have been shown to display intoxication and to develop tolerance and dependence [9], thus becoming good animal models to study the biological determinants predisposing to alcohol abuse and alcoholism in humans. Rat lines developed from the Wistar strain at the University of Chile voluntarily consume ethanol at either low (0.1–2 g ethanol/kg/day: UChA line) or high (4–7 g ethanol/kg/day: UChB line) levels.

Both in humans and rats, mitochondrial aldehyde dehydrogenase (ALDH2) oxidizes acetaldehyde, an aversive metabolite generated in the oxidation of ethanol. In humans, a point mutation in the aldehyde dehydrogenase gene leads to an inactive enzyme (ALDH2*2) [10–12]. Fifteen to 40% of the population of East Asia carry the *ALDH2*2* allele. When these individuals consume ethanol they display five to 20-fold higher blood acetaldehyde levels than subjects who only carry the active *ALDH2*1* allele. Subjects carrying the *ALDH2*2* allele show elevated acetaldehyde levels and marked vasodilation, dysphoria and nausea when consuming ethanol [13]. Heterozygous *ALDH2*2/ALDH2*1* subjects are protected by 66–75% against alcohol abuse and alcoholism, whereas *ALDH2*2/ALDH2*2* homozygous subjects are virtual abstainers [11,14–16]. The possibility that, in humans, the low activity *ALDH2*2* allele may have been coselected to protect subjects against other diseases has been postulated [17].

We recently reported [18] that 94% of the low-drinker Wistar-derived UChA rats code for Arg67 and Glu479

(Arg67/Glu479) in their *Aldh2* gene, whereas none of the high-drinker animals presents such an allele. Rather, 58% of the high-drinker animals present alleles coding for Gln67/Glu479, the most common allele in commercial lines, as first reported by Farres *et al.* [19], and now termed *Aldh2*^{1}. The low-drinker allele Arg67/Glu479 was termed *Aldh2*^{2}. K_m for NAD^+ of ALDH from the homozygous *Aldh2*^{1} and *Aldh2*^{2} animals was 43 μM and 132 μM ($P < 0.001$), respectively, whereas their V_{max} in mitochondrial lysates were 36 nmol NADH/mg protein/min and 28 nmol NADH/mg protein/min ($P < 0.05$), respectively.

Although the association between the genotypes and alcohol consumption phenotypes appears persuasive, in selective breeding studies, the possibility that one allele might have been coselected with other genes determining low consumption cannot be ruled out. Furthermore, when animals are bred for a specific trait, several genes contributing to the same phenotype are selected. Nevertheless, the relevance of a putative gene can be addressed by crossing animals of opposite phenotypes in the selected lines, namely low-drinker and high-drinker F_0 rats, to obtain a heterozygous F_1 generation, and further crossing F_1 generation rats amongst each other to generate F_2 animals. In the latter case, individual genes segregate separately (i.e. in the F_2 generation all genes are 'shuffled'), such that the average F_2 animals should have an intermediate phenotype. These animals can be subsequently specifically genotyped for the alleles of interest; in this case, for the *Aldh2* alleles to determine their possible relevance on voluntary ethanol intake despite a mixed genetic background.

We report studies demonstrating that F_2 rats carrying the *Aldh2*^{1}/*Aldh2*^{1} genotype consume 60% more alcohol than F_2 rats carrying the *Aldh2*^{2}/*Aldh2*^{2} genotype, demonstrating that, even when randomly combined with most other genes, the *Aldh2* genotype determines alcohol consumption in the rat. We have further addressed the mechanism of this action and show that high peak acetaldehyde levels are determined by the *Aldh2*^{2}/*Aldh2*^{2} genotype. The results further suggest that peak acetaldehyde levels rather than steady-state levels determine the aversion to ethanol.

Materials and methods

Animals

Two rat lines derived from the Wistar strain and bred selectively for their alcohol intake, namely UChA (low ethanol-drinkers) and UChB (high ethanol-drinkers), were used through experiments [6].

Animal crossing

Crossing started with F_0 female UChA rats that were *Aldh2*^{2}/*Aldh2*^{2} (GenBank: AY566468), with a voluntary ethanol consumption phenotype of 0.6 ± 0.1 g ethanol/kg/day, and F_0 male UChB rats that were *Aldh2*^{1}/*Aldh2*^{1} (GenBank: AY566467) with a voluntary ethanol consumption phenotype of 5.6 ± 0.8 g ethanol/kg/day ($P < 0.001$) [20]. Animals of the F_1 generation were not phenotyped for ethanol consumption, and thus were alcohol naive. Subsequently, females and males of the F_1 animals were crossed such that no siblings were mated. Rats of the F_2 generation were born from the crossing of F_1 rats. Thus, all rats of the F_2 generation (*Aldh2*^{1}/*Aldh2*^{1}, *Aldh2*^{2}/*Aldh2*^{2} and *Aldh2*^{1}/*Aldh2*^{2}) in this study were derived from matrilineal low-consumption and patrilineal high-consumption phenotype lines [20].

Voluntary ethanol consumption

Two-month-old rats of the F_2 generation were housed in individual cages and offered free choice between a 10% v/v ethanol solution and water from two tubes and rat food *ad libitum*. After 2–3 months, the mean ethanol consumption of the last 30 days was averaged to obtain the mean ethanol consumption for each animal and data were expressed as g ethanol/kg body weight/day.

Genotyping of F_2 animals

After determining ethanol consumption, tail blood was sampled for genotyping of *Aldh2*^{2} and *Aldh2*^{1} alleles according to Sapag *et al.* [18]. Genotyping and alcohol consumption phenotypes were conducted under a double-blind design.

Ethanol and acetaldehyde blood levels

To determine the *in vivo* acetaldehyde blood levels in F_2 generation rats genotyped as *Aldh2*^{2}/*Aldh2*^{2} and *Aldh2*^{1}/*Aldh2*^{1} ethanol (20% v/v solution in saline) was administered intraperitoneally at a dose of 1 g/kg. Ethanol and acetaldehyde levels were measured at 5, 10, 15, 30 and 60 min after ethanol administration in 0.1 ml samples of blood obtained after catheterization of the carotid artery of a previously anaesthetized rat.

Ethanol and acetaldehyde concentrations were determined by head space gas chromatography according to Eriksson *et al.* [21,22].

Statistical analysis

Results were expressed as mean ± SEM. Differences were analysed by Student's *t*-test or analysis of variance as required.

Results

Influence of genotype on voluntary ethanol consumption

Fig. 1 shows the rate of voluntary ethanol consumption of F_0 high-drinker (UChB) and low-drinker (UChA) rats homozygous for two *Aldh2* alleles: *Aldh2*^{1}/*Aldh2*^{1} and *Aldh2*^{2}/*Aldh2*^{2}. As indicated in Methods section, the F_1 generation was naive and did not have access to ethanol.

Homozygous *Aldh2*^{1}/*Aldh2*^{1} rats consumed 65% ($P < 0.01$) more ethanol than *Aldh2*^{2}/*Aldh2*^{2} rats. Heterozygous F_2 *Aldh2*^{1}/*Aldh2*^{2} rats drink as *Aldh2*^{1}/*Aldh2*^{1} rather than as *Aldh2*^{2}/*Aldh2*^{2} rats (Fig. 1).

Influence of genotype on blood acetaldehyde levels

Because *Aldh2*^{1}/*Aldh2*^{1} animals drink differently from *Aldh2*^{2}/*Aldh2*^{2} animals, the levels of blood acetaldehyde attained were determined following a standard dose of alcohol (1 g/kg) administered intraperitoneally to *Aldh2*^{1}/*Aldh2*^{1} and *Aldh2*^{2}/*Aldh2*^{2} (heterozygous *Aldh2*^{1}/*Aldh2*^{2} animals were not studied). Fig. 2 shows that 5 min ($P < 0.0025$) after ethanol administration, *Aldh2*^{2}/*Aldh2*^{2} animals display five-fold higher blood acetaldehyde levels than *Aldh2*^{1}/*Aldh2*^{1} animals and three-fold higher levels at 10 min ($P < 0.01$). There were no significant differences between blood acetaldehyde levels of F_2 *Aldh2*^{2}/*Aldh2*^{2} and *Aldh2*^{1}/*Aldh2*^{1} rats at 15 min whereas the steady-state acetaldehyde levels were identical at 30 and 60 min post-ethanol injection.

Influence of genotype on the rates of ethanol elimination

The overall rate of ethanol elimination from the blood after full ethanol absorption (Table 1) was not significantly different between *Aldh2*^{1}/*Aldh2*^{1} and *Aldh2*^{2}/*Aldh2*^{2} rats, which is in line with the absence of differences in acetaldehyde levels observed under steady-state conditions in these two F_2 lines.

Discussion

Data presented show that the genotype for mitochondrial aldehyde dehydrogenase greatly influences voluntary ethanol consumption in this animal model, without significantly affecting the overall rates of ethanol metabolism. The higher affinity (for NAD^+) and higher activity (V_{max}) of *Aldh2*^{1}/*Aldh2*^{1} rats is associated with a 65% greater ethanol intake than in *Aldh2*^{2}/*Aldh2*^{2} rats.

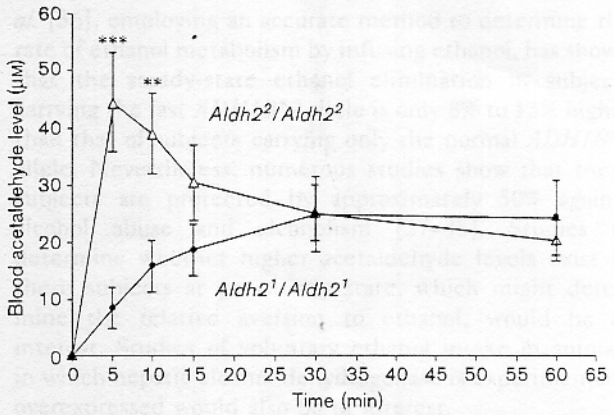
Previous studies [23] have shown that the steady-state levels of blood acetaldehyde (measured 30–90 min after ethanol administration) are not different in F_0 UChA (low drinkers which are mostly *Aldh2*^{2}/*Aldh2*^{2}) compared to those in UChB (high drinkers, in a high proportion *Aldh2*^{1}/*Aldh2*^{1}). This was confirmed in the present study. However, these data do not explain the mechanism by which a less active ALDH2 in the UChA rats is associated with a lower ethanol consumption. Given the relevance of the *Aldh2* genotype on the ethanol consumption phenotype (Fig. 1), we tested the hypothesis that rats with the *Aldh2*^{1}/*Aldh2*^{1} and *Aldh2*^{2}/*Aldh2*^{2} genotypes, even if not showing differences in steady-state blood acetaldehyde

Table 1 ***In vivo*** **rate of ethanol metabolism by F_2 hybrid rats genotyped as *Aldh2*^{1}/*Aldh2*^{1} or *Aldh2*^{2}/*Aldh2*^{2}**

Genotype	Blood ethanol disappearance (mg/per 100 ml of blood/h)	Rate of ethanol metabolism (mmol/kg/h)
Aldh2^1/*Aldh2*^{1}	59.0 ± 10.3 (4)	9.55 ± 0.26 (4)
Aldh2^2/*Aldh2*^{2}	65.2 ± 6.8 (4)	9.05 ± 0.99 (4)

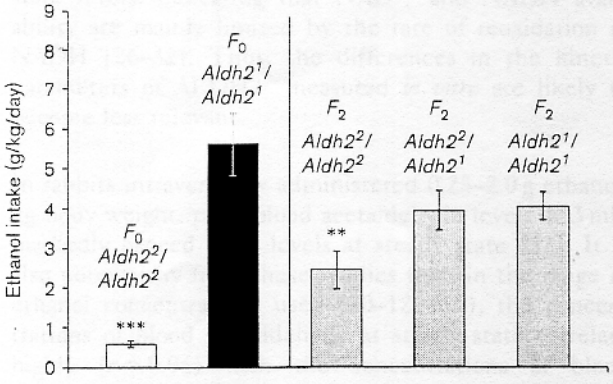
A dose of ethanol of 1 g/kg was administered intraperitoneally at time zero and ethanol concentration was determined in blood samples obtained from the carotid artery. The number of animals is shown in parenthesis. Rates of ethanol metabolism per unit body weight were calculated from the concentration of ethanol in blood at time zero (intercept of the linear part of the curve) which provides an estimate the volume of distribution for ethanol (ml blood per kg of body weight) and the rate of ethanol disappearance from blood.

Fig. 2



Blood acetaldehyde concentrations after the administration of ethanol (1 g/kg) to F_2 hybrid rats genotyped as *Aldh2*^{2}/*Aldh2*^{2} or *Aldh2*^{1}/*Aldh2*^{1}. Significant differences in blood acetaldehyde levels: ***$P<0.0025$, **$P<0.01$.

Fig. 1



Ethanol consumption of F_0 and F_2 rats according to their *Aldh2* genotype. Bars represent the mean ± SEM of ethanol consumption expressed as g ethanol/kg body weight/day (F_0 low-drinkers: UchA, F_0 high-drinkers: UchB; ***$P<0.001$). Homozygous F_2 *Aldh2*^{1} rats consumed 65% more ethanol than F_2 homozygous *Aldh2*^{2} (**$P<0.01$) or heterozygous *Aldh2*^{1}/*Aldh2*^{2} rats (**$P<0.05$).

levels, would present significant differences in acetaldehyde levels immediately upon metabolizing ethanol, with higher peak blood acetaldehyde levels in *Aldh2²/Aldh2²* than in *Aldh2¹/Aldh2¹* animals. The data obtained fully supported such a hypothesis; peak blood acetaldehyde levels achieved in *Aldh2²/Aldh2²* were three- to five-fold higher than peak levels in *Aldh2¹/Aldh2¹* animals, whereas we confirmed earlier observations that the steady-state levels of acetaldehyde were identical.

It is of interest that, in Asians carrying the Glu487Lys allele of *ALDH2*, an aversion to alcohol following moderate alcohol drinking, shown by their refusal to continue drinking, occurred only at the earliest time studied (30 min) but not subsequently (60 min), despite identical high blood acetaldehyde levels at both times [13]. It should be noted that the Glu487Lys mutation (*ALDH2*2*) in humans leads to a greater inhibition of enzyme activity [10,12] compared to the Arg67Gln mutation (*Aldh2²*) in the rat [18]. Despite this, it appears that the initial levels of acetaldehyde in both species determine the aversion to ethanol.

The differences in peak acetaldehyde levels in animals with ALDH2 with different affinities for NAD^+ may reflect the initial availability of NAD^+ in the cell. Before the presentation of ethanol to the liver, the redox state ($NADH/NAD^+$) of this organ is in an oxidized state, as shown by a low lactate/pyruvate ratio and a low β-hydroxybutyrate/acetoacetate ratio [24,25]. This would allow the differences in kinetic constants of $ALDH2^2$ to be initially expressed; namely, a lower affinity for NAD^+ and a lower V_{max} $ALDH2^2$ would be reflected in lower amounts of acetaldehyde oxidized to acetate, and thus in higher peak acetaldehyde levels. On the other hand, the lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios increase when ethanol metabolism occurs at steady-state levels, indicating that NAD^+ and NADH availability are mainly limited by the rate of reoxidation of NADH [26–32]. Thus, the differences in the kinetic parameters of $ALDH2^2$ measured *in vitro* are likely to become less relevant.

In rabbits intravenously administered 0.25–2.0 g ethanol/kg body weight, peak blood acetaldehyde levels at 3 min markedly exceed their levels at steady state [33]. It is also noteworthy from these studies that, in the range of ethanol concentrations used (20–120 mM), the concentrations of blood acetaldehyde at steady state correlate highly ($r = 0.96$) with the concentrations of blood ethanol, suggesting that the alcohol dehydrogenase reaction is in quasi equilibrium in steady-state conditions.

We propose that it is the higher initial peak of acetaldehyde levels in *Aldh2²/Aldh2²* rats that determines a conditioned aversion to ethanol. Such a view might partly explain the paradoxical findings of Isse *et al.* [34] who found that knockout *Aldh2−/−* mice consume 75% less ethanol than wild-type mice, despite not showing significant steady-state differences in blood acetaldehyde levels. It should be noted that although the mouse has a very active cytosolic aldehyde dehydrogenase [35] that could take over the oxidation of acetaldehyde, it is also expected to be limited by the rate of NADH reoxidation. Determination of acetaldehyde at early times in these animals might shed more light on this matter.

Another paradoxical result, which may be understood better in light of the present findings, is the fact that although some humans present a 10-fold more active alcohol dehydrogenase (*ADH2*2* or *ADH1B*2*) there are no reports of higher blood acetaldehyde levels (at steady state) in them. Furthermore, recent work by Neumark *et al.* [36], employing an accurate method to determine the rate of ethanol metabolism by infusing ethanol, has shown that the steady-state ethanol elimination in subjects carrying the fast *ADH1B*2* allele is only 8% to 13% higher than that of subjects carrying only the normal *ADH1B*1* allele. Nevertheless, numerous studies show that these subjects are protected by approximately 50% against alcohol abuse and alcoholism [37–45]. Studies to determine whether higher acetaldehyde levels exist in these subjects at pre-steady state, which might determine the relative aversion to ethanol, would be of interest. Studies of voluntary ethanol intake in animals in which hepatic alcohol dehydrogenase is experimentally overexpressed would also be of interest.

In addition to the pharmacogenetic implications of the present findings, which were made feasible by breeding methodologies that cannot be carried out in humans, the data obtained suggest that an aversion to ethanol could be therapeutically achieved without increases in steady-state acetaldehyde levels, but only short-lived increases in peak acetaldehyde levels. For patients who continue consuming alcohol despite such an intervention, the effects of an acetaldehyde elevation in extrahepatic tissues would be minimal.

Overall, in an animal model, we have shown that *Aldh2* polymorphisms play an important role in determining peak blood acetaldehyde levels that may modulate voluntary ethanol consumption. We postulate that the markedly higher peak acetaldehyde levels that develop in *Aldh2²/Aldh2²* animals are aversive and lead these animals to reduce their alcohol intake compared to that of *Aldh2¹/Aldh2¹* animals.

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