Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats

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ABSTRACT **Animals selectively bred for a desirable trait retain wanted genes but exclude genes that may counteract the expression of the former. The possible interactions between selected and excluded genes cannot be readily studied in transgenic or knockout animals but may be addressed by crossing animals bred for opposite traits and studying the F_2 offspring. Ninety-seven percent of Wistar-derived rats selectively bred for their voluntary low-alcohol consumption display a mutated nuclear allele of aldehyde dehydrogenase *Aldh2*^{2} that encodes an enzyme with a low affinity for NAD$^+$, whereas rats bred for high-alcohol consumption do not present the *Aldh2*^{2} allele. This enzyme is inserted into mitochondria, where NADH-ubiquinone oxidoreductase (complex I) regenerates NAD$^+$. The possible influence of complex I on ALDH2 activity and voluntary ethanol intake was investigated. Homozygous *Aldh2*^{2}/*Aldh2*^{2} rats derived from a line of high-drinker F_0 females (and low-drinker F_0 males) showed a markedly higher ethanol consumption (3.9±0.5 g·kg^{-1}·day^{-1}) than homozygous animals derived from a line of low-drinker F_0 females (and high-drinker F_0 males) (1.8±0.4 g·kg^{-1}·day^{-1}). Mitochondria of F_2 rats derived from high alcohol-consuming females were more active in oxidizing substrates that generate NADH for complex I than were mitochondria derived from low alcohol-consuming females, leading in the former to higher rates of acetaldehyde metabolism and to a reduced aversion to ethanol. This is the first demonstration that maternally derived genes can either allow or counteract the phenotypic expression of a mutated gene in the context of alcohol abuse or alcoholism.—Quintanilla, M. E., Tampier, L., Valle-Prieto, A., Sapag, A., Israel, Y. Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats.**

Key Words: alcoholism · penetrance · mitochondria · mtDNA · polymorphism

A NUMBER OF STUDIES have shown that 50–60% of the predisposition to develop alcoholism is determined by genetic factors (1–3). Several groups have selectively bred animals for their voluntary low- or high-ethanol consumption. Initial studies were those by Mardones et al. (4), followed by studies by Eriksson et al. (5), Li et al. (6), Colombo et al. (7), and Le et al. (8). 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.

Both in humans and rats, 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 5- to 20-fold higher blood levels of acetaldehyde than subjects who only carry the active *ALDH2*1* allele. Subjects carrying the *ALDH2*2* allele show 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, while *ALDH2*2/ALDH2*2* homozygous subjects are virtual abstainers (11, 14–16).

Recently, a mutation in the *Aldh2* gene (lower case nomenclature used for rodents) was shown to strongly segregate with voluntary ethanol consumption in Wistar-derived rats (17). Low alcohol-consuming (UChA) rats display a point mutation in the *Aldh2* gene that changes glutamine-67 (*Aldh2*^{1} allele) into arginine-67 (*Aldh2*^{2} allele) in the enzyme coded. The *Aldh2*^{2} allele was found in 97% of low-drinker rats (UChA) but was always absent from high-drinker rats

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(UChB). The high-drinker line (in addition to *Aldh2*[1]) carried a third allele (*Aldh2*[3]) in 42% of the animals. The *Aldh2*[3] was never present in low drinkers. Thus, *Aldh2*[2] and *Aldh2*[3] are specific for low and high drinkers, respectively, in these lines. The *Aldh2*[3] allele codes for arginine-67 but also contains a second point mutation that changes glutamic-479 into lysine-479 (17). These point mutations in rat *Aldh2* are different from those in human *ALDH2* (Glu-487 into Lys-487) (18, 19).

The ALDH2 of rats homozygous for the *Aldh2*[2] allele shows a K_m for NAD^+ 4- to 5-fold higher than those for ALDH2 s of rats homozygous for *Aldh2*[3] or *Aldh2*[1] (17). Such a difference might constitute a mechanism leading to low ethanol consumption due to lower rates of acetaldehyde metabolism.

Although mammalian ALDH2 is coded by a nuclear gene, the enzyme is inserted into mitochondria, where the NADH generated in the oxidation of acetaldehyde is reoxidized back to NAD^+ by complex I (NADH-ubiquinone oxidoreductase) of the respiratory chain. Thus, the in vivo ability to metabolize acetaldehyde depends not only on the kinetic properties of ALDH2, but on the ability of mitochondrial complex I to reoxidize the NADH generated to NAD^+. Complex I is formed by proteins encoded by nuclear (autosomal and X chromosome) genes and mitochondrial (maternally transmitted) genes (20–23).

When animals are selectively bred for a desirable trait, the animals not only retain wanted genes but also exclude genes that may counteract the expression of the former. Incomplete penetrance of a gene may result when opposing genes present in the original pool are recovered, for example as the result of mating with animals from the original stock or with animals of opposite traits. The existence of epistasis, in which one gene abolishes the phenotypic expression of another gene (24), likely explains the fact that associations observed between polymorphisms and a specific disease in one population are often not observed in other populations (see ref 25). These interactions are difficult to investigate in transgenic or knockout animals, but can be addressed by crossing animals bred for opposite traits (e.g., high alcohol consumers and low alcohol consumers) and studying the F_2 offspring for their phenotype and their genetic and biological characteristics. The aim of this study was to determine whether in homozygous *Aldh2*[2]/*Aldh2*[2] rats of the F_2 generation derived from the two extreme phenotypes of alcohol consumption, Complex I activity influences *1)* acetaldehyde oxidation by intact mitochondria, *2)* in vivo acetaldehyde metabolism, and *3)* voluntary ethanol consumption. Studies presented here show that differences in complex I activity of F_2 rats either allow or fully abolish the phenotypic expression of the *Aldh2*[2]/*Aldh2*[2] genotype on the variables indicated above. These studies constitute the first demonstration of the role of the mitochondrial electron transport ability on voluntary ethanol consumption and the relevance of maternal effects on the penetrance of a polymorphism in the area of alcohol abuse and alcoholism.

MATERIALS AND METHODS

Animals

Two rat lines derived from the Wistar strain and bred selectively for their alcohol intake were used in these studies (4). In the founder colony, one male was initially mated with two females (Fig. 1 of ref 4), which allows mitochondrial diversity. The high-drinker and low-drinker lines, kept under selective breeding for more than 50 years, show marked differences in their voluntary ethanol consumption (10% v/v ethanol and water offered ad libitum). Phenotypes are low drinkers (UChA, virtual abstainers) and high drinkers (UChB, bibulous).

Animal intercrossing

Crossings started with twelve F_0 rats: six UChA rats that were homozygous *Aldh2*[2]/*Aldh2*[2] (GenBank: AY566468), with a voluntary ethanol consumption phenotype of 0.7 ± 0.4 g ethanol·kg^{-1}·day^{-1} (means±SE) and six UChB rats that were homozygous for *Aldh2*[3]/*Aldh2*[3] (GenBank: AY566469) with a 6-fold difference in voluntary ethanol consumption phenotype of 4.4 ± 0.5 g ethanol·kg^{-1}·day^{-1} ($P<0.001$). The F_1 generation comprised 44 offspring, which were not phenotyped for alcohol intake and thus were alcohol naive. Subsequently, F_1 animals were crossed such that no siblings were mated. Rats of the F_2 generation born from the crossing of F_1 animals were 108 animals, which can be grouped according to their maternal line origin (**Fig. 1**):

1) Group #1 comprised F_2 rats (males and females) born to a F_1 hybrid mother that was the offspring of a F_0 low-drinker mother (and a F_0 high-drinker father). Group #1 had 26 ♀ and 36 ♂ F_2 rats. For simplicity these animals are referred to as derived from the "UChA low-drinker maternal line."

2) Group #2 comprised F_2 rats (males and females) born to a F_1 hybrid mother that, in turn, was the offspring of a F_0 high-drinker mother (and a low-drinker father). Group #2 had 18 ♀ and 28 ♂ F_2 rats. For simplicity these animals are referred to as derived from the "UChB high-drinker maternal line."

Voluntary ethanol consumption

Two-month-old rats of the F_2 generation were housed in individual cages and permanently offered the choice of a 10% v/v ethanol solution or water from two tubes and food ad libitum. After 2–3 months, when ethanol and water preferences stabilize, the mean ethanol consumption of the last 30 drinking days was averaged to obtain the mean ethanol consumption for each animal, and expressed as g ethanol·kg body weight^{-1}·day^{-1}.

Genotyping of F_2 animals

After determining voluntary ethanol consumption, rat tail blood was sampled for genotyping of *Aldh2* alleles (*Aldh2*[2], GenBank: AY566468, and *Aldh2*[3], GenBank: AY566469) according to Sapag et al. (17). Genotyping and alcohol consumption phenotypes were conducted under a double blind design.

Acetaldehyde disappearance rates

To determine the in vivo rate of acetaldehyde metabolism in hybrid F_2 rats showing *Aldh2*[2]/*Aldh2*[2] and *Aldh2*[3]/*Aldh2*[3] genotypes in groups #1 and #2 above, acetaldehyde (75

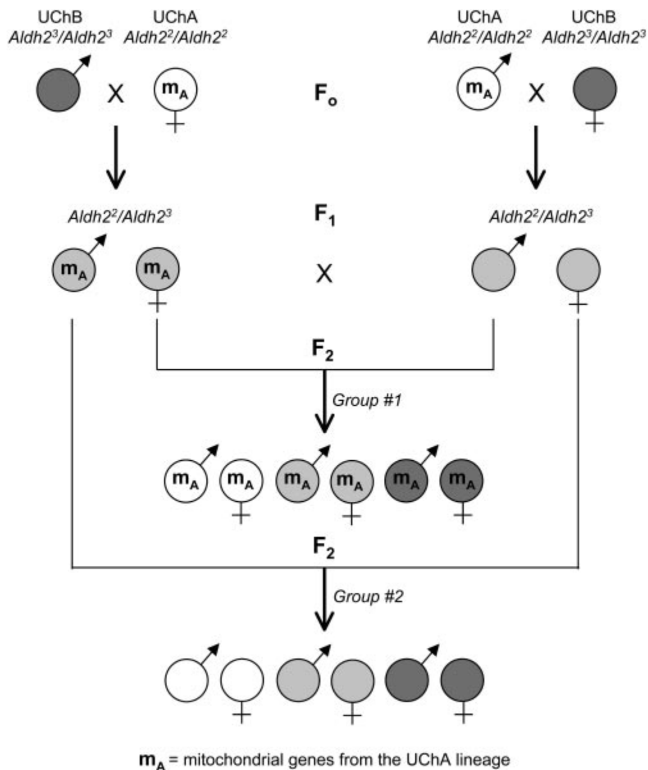

Figure 1. Development of an F_2 generation of rats from original F_0 lines UChA (low drinker) and UChB (high drinker). Crossings started with 12 F_0 rats: six UChA rats that were homozygous for *Aldh2*^{2} (GenBank: AY566468) and six UChB rats that were homozygous for *Aldh2*^{3} (GenBank: AY566469). We obtained 44 offspring in the F_1 generation. These animals were not phenotyped for alcohol intake and thus were alcohol naive. Subsequently, F_1 animals were crossed such that no siblings were mated. Hybrid F_2 rats born from F_1 crossings were 108 animals in which two groups can be differentiated according to the maternal line: group #1 from a F_0 maternal line of low-drinker UChA and group #2 from a F_0 maternal line of high-drinker UChB. The insert m_A indicates that the animal carries mitochondrial genes from the UChA lineage.

mg/kg) was administered intraperitoneally as a 1.6% (w/v) solution in saline. Acetaldehyde levels were measured 2, 4, and 6 min after its administration in 0.1 mL samples of blood obtained from the superior sagittal blood sinus of previously anesthetized rats. The concentration of acetaldehyde was determined in whole blood by head space gas chromatography, according to Eriksson et al. (26). An ethanol peak was not found in any blood sample chromatogram, indicating that acetaldehyde was not reduced to ethanol by the alcohol dehydrogenase system.

Study of mitochondrial function

Mitochondrial acetaldehyde disappearance rates and mitochondrial O_2 uptake were determined with acetaldehyde (0.1 mM) as substrate. Two additional substrates were used, glutamate (10 mM) and succinate (10 mM), which contribute electrons to complex I and complex II, respectively. The rate of O_2 uptake was measured after addition (state 3) and exhaustion (state 4) of ADP. Liver mitochondria were prepared as described by Gil et al. (27). Mitochondrial O_2 uptake was determined polarographically by the use of a Clark electrode as described previously (28). Acetaldehyde disappearance rates in mitochondria were determined at 36°C as described by Hasumura et al. (29). Acetaldehyde was measured at 0, 2, 4, 8, 10, and 12 min of incubation by head space gas chromatography.

Statistical analysis

Results were expressed as means ± SE. Differences were analyzed by Student's *t* test.

RESULTS

Genotyping of animals for *Aldh2*

Of 108 rats from the F_2 generation, 28 rats showed the *Aldh2*^{2}/*Aldh2*^{2} genotype, 52 rats were *Aldh2*^{2}/*Aldh2*^{3} and 28 rats were *Aldh2*^{3}/*Aldh2*^{3}, showing a Mendelian pattern of inheritance for *Aldh2* alleles. In group #1 (low-drinker maternal line): 12 rats were genotyped as *Aldh2*^{2}/*Aldh2*^{2}; 38 rats as *Aldh2*^{2}/*Aldh2*^{3} and 12 rats as *Aldh2*^{3}/*Aldh2*^{3}. In group #2 (high-drinker maternal line), 16 rats were genotyped as *Aldh2*^{2}/*Aldh2*^{2}; 14 as *Aldh2*^{2}/*Aldh2*^{3} and 16 rats as *Aldh2*^{3}/*Aldh2*^{3}.

Alcohol consumption of F_2 animals of different *Aldh2* genotypes and maternal lines

The voluntary ethanol consumption of the complete cohort of F_2 hybrid rats was compared with the two original F_0 lines. The F_2 rats consumed higher amounts of ethanol (2.6±0.35 g/ethanol·kg^{-1}·day^{-1}) than the original low-drinker F_0 UChA line (0.7±0.4 g ethanol·kg^{-1}·day^{-1}) and lower amounts of ethanol than the original high-drinker F_0 UChB line (4.4±0.5 g ethanol·kg^{-1}·day^{-1}), with a marked variation between individual animals. Among these, homozygous F_2 *Aldh2*^{2}/*Aldh2*^{2} rats in group #2 derived from high-drinker F_0 females (and low-drinker F_0 males) showed markedly higher ($P<0.005$) ethanol consumption (3.9±0.5 g·kg^{-1}·day^{-1}) than F_2 homozygous *Aldh2*^{2}/*Aldh2*^{2} rats of group #1 derived from low-drinker F_0 females (and high-drinker F_0 males) (1.8±0.4 g·kg^{-1}·day^{-1}) (**Fig. 2**). In F_2 rats carrying the *Aldh2*^{3}/*Aldh2*^{3} genotype, the maternal line did not influence alcohol consumption; alcohol consumption of group #1 *Aldh2*^{3}/*Aldh2*^{3} rats was virtually identical to that of group #2 *Aldh2*^{3}/*Aldh2*^{3} rats (Fig. 2). For heterozygous *Aldh2*^{2}/*Aldh2*^{3} rats the influence of the maternal line on ethanol consumption was small and did not reach statistical significance (Fig. 2).

Data obtained indicate a strong potentiation of the *Aldh2*^{2}/*Aldh2*^{2} genotype and other gene(s) transmitted through the maternal line. Due to the mitochondrial location of ALDH2, a possible explanation was a change in the maternally transmitted ability of complex I to reoxidize NADH. Thus, we investigated whether F_2 *Aldh2*^{2}/*Aldh2*^{2} rats arising from either the low or high consumption maternal lines (groups #1 and #2) differed with respect to *1)* oxidation of acetaldehyde by isolated mitochondria, *2)* mitochondrial O_2 consump-

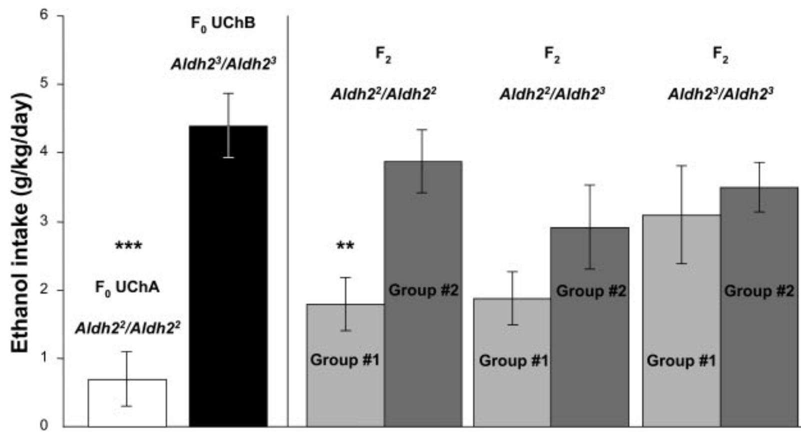

Figure 2. Ethanol consumption of F_0 and F_2 rats. Bars represent means ± SE of ethanol consumption (g ethanol·kg body weight^{-1}·day^{-1}) with regard to the original line (UChA or UChB), the *Aldh2* genotype, and the maternal ethanol consumption line. Group #1: F_2 from a F_0 low-consumption UChA maternal line (and a F_0 high-consumption UChB father). Group #2: F_2 from a F_0 high-consumption UChB maternal line (and a F_0 low-consumption UChA father). The maternal line greatly influenced ($P<0.005$) alcohol consumption of $Aldh2^2/Aldh2^2$ but did not influence the consumption of $Aldh2^3/Aldh2^3$ rats. The maternal line did not significantly alter ethanol consumption of heterozygous $Aldh2^2/Aldh2^3$ rats (**$P<0.005$; ***$P<0.001$).

tion with substrates that provide electrons to complex I of the mitochondrial respiratory chain, such as acetaldehyde and glutamate, *3)* mitochondrial oxygen consumption when the substrate was succinate, an electron donor at the complex II site, and *4*) blood acetaldehyde disappearance after its in vivo administration.

Mitochondrial function

Studies were conducted in F_2 rats genotyped as $Aldh2^2/Aldh2^2$ of groups #1 and #2 to determine the rate of acetaldehyde oxidation by intact rat liver mitochondria in either the absence (state 4) or presence (state 3) of ADP. As can be seen (**Table 1**), the rate of acetaldehyde metabolism was significantly lower in mitochondria of rats of group #1 (UChA low-drinker maternal line) than in mitochondria of rats of group #2 (UChB high-drinker maternal line) in states 4 and 3. The same group differences were observed for O_2 uptake in state 3 with glutamate as substrate, which contributes NADH for complex I of the mitochondrial respiratory chain (**Table 2**). Conversely, no maternal line differences in oxygen consumption were observed with succinate, a mitochondrial complex II substrate (Table 2).

Acetaldehyde disappearance rate

An intraperitoneal dose of acetaldehyde of 75 mg/kg was administered to F_2 hybrid rats of groups #1 and #2 genotyped either as homozygous $Aldh2^2/Aldh2^2$ or $\mathrm{Aldh2}^3/\mathrm{Aldh2}^3$ ($Aldh2^2/Aldh2^3$ animals were not studied). Direct administration of acetaldehyde avoids possible changes in the generation of acetaldehyde that may occur when ethanol itself is administered. **Table 3** shows that F_2 $Aldh2^2/Aldh2^2$ animals in group #1 (low-drinker maternal line) display a significantly lower rate of acetaldehyde disappearance than F_2 $Aldh2^2/Aldh2^2$ rats of group #2 (high-drinker maternal line) and a lower rate than F_2 rats genotyped as $Aldh2^3/Aldh2^3$ of groups #1 and #2. Since the rate of acetaldehyde disappearance is related to the rate of electron flow in the respiratory chain (31), these data again suggest that in F_2 $Aldh2^2/Aldh2^2$ rats the rate of acetaldehyde metabolism in vivo is limited by the ability of intact mitochondria to regenerate NAD^+ from NADH at complex I.

DISCUSSION

When animals are selectively bred for a specific trait several genes contributing to a single phenotype (e.g., low consumption) may be selected. In the same process, genes that may abolish the phenotype are excluded from the genetic pool. Epistasis is the condition in which the phenotypic expression of one gene is fully suppressed by the expression of another gene (24, 25). In the present studies, we bred an F_2 generation that brings together in a single individual genes that existed in the original stock, namely genes present in the F_0 low-drinker UChA and the F_0 high-drinker UChB rats. A random segregation of genes that determine low and

TABLE 1. *Acetaldehyde oxidation by intact liver mitochondria from F_2 $Aldh2^2/Aldh2^2$ rats arising from F_0 UChA females (group #1) and F_0 UChB females (group #2)*[a]

		Acetaldehyde disappearance rate (nmol/mg protein/min)		O_2 uptake rate (*n*-atoms/mg protein/min)	
		Group #1	Group #2	Group #1	Group #2
State 4	(n = 3)	5.82 ± 0.38***	9.53 ± 1.18	6.29 ± 0.87	6.64 ± 0.60
State 3	(n = 3)	15.05 ± 0.98**	20.02 ± 0.54	21.70 ± 0.20**	28.30 ± 1.84

[a] Acetaldehyde (0.1 mM) was added as substrate. The number of animals is indicated in parentheses. Significant differences between groups #1 and #2: ** $P < 0.025$; *** $P < 0.005$.

TABLE 2. *Mitochondrial respiration and oxidative phosphorylation of isolated liver mitochondria from F_2 Aldh2^2/Aldh2^2 rats arising from F_0 UChA females (group #1) and F_0 UChB females (group #2) for glutamate and succinate as substrates*[a]

	Glutamate		Succinate	
	Group #1	Group #2	Group #1	Group #2
(*a*) State 4 respiration (*n*-atoms O_2/mg protein/min)	8.28 ± 0.75*	10.38 ± 0.62	15.27 ± 1.07	17.82 ± 1.48
(*b*) State 3 respiration (*n*-atoms O_2/mg protein/min)	35.63 ± 2.20**	45.40 ± 2.68	83.82 ± 1.62	88.71 ± 2.15
(*c*) Respiratory control index (*b*/*a*)	4.39 ± 0.58	4.37 ± 0.12	5.55 ± 0.34	5.06 ± 0.27
(*d*) ADP/O	2.51 ± 0.17	2.57 ± 0.29	1.79 ± 0.04	1.79 ± 0.04

[a] Values are means ± SE of 4 experiments. In each experiment one rat was used. Significant differences between groups #1 and #2: * $P < 0.05$; ** $P < 0.025$.

high ethanol consumption is expected to occur in animals of the F_2 generation. Indeed, average ethanol consumption phenotype of F_2 rats is in the middle of the two extreme phenotypes of F_0 animals. In F_2 *Aldh2^2/Aldh2^2* animals alcohol consumption was markedly influenced by the alcohol consumption phenotype inherited through the F_0 maternal line, suggesting the existence of genetic factors present in the X chromosome or the maternal mitochondria that either allow or abolish the phenotypic expression of the *Aldh2^2/Aldh2^2* genotype. The possible influence of the Y chromosome was discarded given the fact that a lower ethanol consumption was observed in *Aldh2^2/Aldh2^2* rats of group #1, group derived from the high-drinker paternal line.

The *Aldh2^2/Aldh2^2* genotype correlated with a low alcohol consumption phenotype only when ALDH2^2 was inserted into mitochondria having a reduced ability to reoxidize NADH by complex I, and thus a reduced ability to replenish NAD^+ for ALDH2^2. A reduced ability to regenerate NAD^+ is expected to have a greater influence on the lower affinity ALDH2^2 (high K_m for NAD^+) than on the higher affinity ALDH2^3 (low K_m for NAD^+). This was indeed the case: mitochondria from the maternal low-drinker line did not influence voluntary ethanol intake of F_2 rats with the *Aldh2^3/Aldh2^3* genotype.

Mitochondria derived from the low-drinker maternal line showed significantly lower rates of O_2 consumption with acetaldehyde and glutamate as substrates (which enter electrons at the NADH:ubiquinone reductase or complex I), but were normal when succinate was used as substrate (a complex II substrate). Mitochondria of animals derived from a low-drinker UChA maternal line (and a high-drinker UChB father) and carrying the *Aldh2^2/Aldh2^2* genotype showed lower rates of acetaldehyde metabolism than *Aldh2^2/Aldh2^2* animals derived from the high-drinker UChB maternal line. The maternal line did not influence the in vitro or in vivo acetaldehyde metabolism of F_2 rats carrying the *Aldh2^3/Aldh2^3* genotype.

TABLE 3. *In vivo rate of acetaldehyde metabolism by F_2 rats according to genotype and maternal group origin*[a]

Genotype	Maternal group	Acetaldehyde metabolism (mmol/kg/h)	*P* vs. (*a*)
Aldh2^2/Aldh2^2	(*a*) Group #1	14.10 ± 0.48 (6)	
	(*b*) Group #2	17.23 ± 0.99 (6)	$P < 0.01$
Aldh2^3/Aldh2^3	(*c*) Group #1	16.93 ± 0.83 (3)	$P < 0.01$
	(*d*) Group #2	17.05 ± 0.74 (3)	$P < 0.005$

[a] A dose of acetaldehyde of 75 mg/kg was administered at time zero and rates of acetaldehyde disappearance were calculated for each animal by determining the intercept at a concentration of acetaldehyde of zero. The number of animals is shown in parentheses.

Many genes exist that are either permissive or protective for the development of alcoholism in humans (3). In rats, breeding studies have allowed a relative estimate of the number of genes associated with high- and low drinking. Le and co-workers (8), who started their breeding program with the highly heterogeneous strain developed at the National Institutes of Health from the crossing of eight different rat strains (30), showed that ethanol voluntary consumption in the high-drinking line increased steadily after each crossing for seven or eight generations, confirming the existence of several permissive (and likely additive) genes in the original stock. On the other hand, the low-drinking line stabilized its ethanol consumption in only two generations, indicating the existence of a smaller number of potent protective genes. The fact that mitochondrial genes pass almost exclusively from the mother to all offspring (31) may add to the rapidity of acquisition of the low consumption phenotype. This observation is in agreement with reports by Mardones and Segovia-Riquelme (4) for the Wistar-derived rat lines used in the present study and by Li and co-workers, who started their selective breeding with the heterogeneous NIH line (T.-K. Li, personal communication). From the data obtained in our studies, it can be estimated that the combination of the *Aldh2^2/Aldh2^2* genotype plus a low activity mitochondrial complex I accounts for 50–60% of the difference in ethanol intake between the original UChA and UChB lines, which were kept for decades under selective breeding for their differences in ethanol intake.

In humans, an example of gene addition leading to a reduced ethanol consumption is seen in East Asians, a population with the highest prevalence of a high-activity alcohol dehydrogenase (ADH1*B; formerly ADH2-2) and an inactive ALDH2 (ALDH2*2) (32, 33). The combination of these factors has been shown to have an additive effect in protecting subjects against

alcoholism (34, 35). Lin and Cheng (36) have hypothesized that the *ALDH2*2* allele was selected as a protective mechanism against mortality due to the combined effects of a high ethanol consumption and hepatitis B, in a geographic area with a high prevalence of the latter condition. The explanation may hold for the high prevalence of ADH1*B. The possibility that abnormalities in mitochondrial genes that control the rate of electron flow may have been selected in some populations is worth investigating. An analysis of mitochondrial DNA in alcoholics in Japan showed a 491 bp deletion (as heteroplasmy) in the ATPase gene, coded by mtDNA, in 58% of cases, while this deletion was not observed in controls (37). An abnormal ATPase may lead to an abnormal coupling of oxidation and phosphorylation, resulting in an increased electron flow though the respiratory chain, thus facilitating NADH and acetaldehyde oxidation. In such a case, protection due to circulating acetaldehyde would be abrogated. While alcohol consumption per se may lead to various mutations and heteroplasmy in mitochondrial DNA (38), the probability that the same 491 bp deletion could have resulted from alcohol consumption in all cases is highly unlikely.

The present studies do not allow us to conclude whether the maternal transmission of the mitochondrial abnormalities in complex I is of X chromosomal or mitochondrial genetic origin, although it is known that direct genetic transmission of mitochondrial DNA occurs only through the maternal line. The possibility of an immediate ethanol-related genomic imprinting was likely avoided by not allowing alcohol access to the F_1 rat generation. More likely, the mitochondrial abnormalities observed in complex I in low-drinker UChA rats were selected in the very process of breeding for the low-ethanol consumption phenotype. Mitochondrial complex I is a multiunit protein formed by proteins coded by nuclear genes and mitochondrial genes (20–23). The small (16 kb) circular mitochondrial DNA encodes seven protein units of the multiunit complex I (21). The full genome of rat mtDNA is known, which should facilitate its sequencing in animals derived from UChA and UChB maternal lines. An additional X chromosome gene encoding the MWFE polypeptide is required for the assembly of all the proteins that form complex I (22).

Overall, our data show that a lower mitochondrial (complex I) ability to reoxidize NADH, transmitted through the maternal line, potentiates the effects of a mutation in *Aldh2* that codes an $ALDH2^2$ with a lower affinity for NAD^+. The combination of both factors strongly segregates with a low-ethanol consumption phenotype in rats. FJ

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