Genetic Polymorphism of Alcohol Dehydrogenase in Europeans: The *ADH2*2* Allele Decreases the Risk for Alcoholism and Is Associated With *ADH3*1*

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Polymorphism at the *ADH2* and *ADH3* loci of alcohol dehydrogenase (ADH) has been shown to have an effect on the predisposition to alcoholism in Asian individuals. However, the results are not conclusive for white individuals. We have analyzed the ADH genotype of 876 white individuals from Spain (n = 251), France (n = 160), Germany (n = 184), Sweden (n = 88), and Poland (n = 193). Peripheral blood samples from healthy controls and groups of patients with viral cirrhosis and alcohol-induced cirrhosis, as well as alcoholics with no liver disease, were collected on filter paper. Genotyping of the *ADH2* and *ADH3* loci was performed using polymerase chain reaction-restriction fragment length polymorphism methods on white cell DNA. In healthy controls, *ADH2*2* frequencies ranged from 0% (France) to 5.4% (Spain), whereas *ADH3*1* frequencies ranged from 47.6% (Germany) to 62.5% (Sweden). Statistically significant differences were not found, however, between controls from different countries, nor between patients with alcoholism and/or liver disease. When all individuals were grouped in nonalcoholics (n = 451) and alcoholics (n = 425), *ADH2*2* frequency was higher in nonalcoholics (3.8%) than in alcoholics (1.3%) (*P* = .0016), whereas the *ADH3* alleles did not show differences. Linkage disequilibrium was found between *ADH2* and *ADH3*, resulting in an association of the alleles *ADH2*2* and *ADH3*1*, both coding for the most active enzymatic forms. In conclusion, the *ADH2*2* allele decreases the risk for alcoholism, whereas the *ADH2*2* and *ADH3*1* alleles are found to be associated in the European population. (Hepatology 2000;31:984-989.)

Ingested alcohol is mostly metabolized in the liver by the successive action of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Both enzymes exhibit genetic polymorphisms that influence the rate of conversion of ethanol to acetaldehyde, and of acetaldehyde to acetate. It has been consistently reported that *ALDH2* is the most important alcohol-metabolizing gene affecting predisposition to alcoholism in Asian populations. The prevalence of the *ALDH2*2* allele, which codes for a physiologically inactive mitochondrial ALDH form, is lower in alcoholics than in nonalcoholics.[1-20] However, this allele has not been found in white individuals.[21]

Regarding ADH, polymorphism is detected at the *ADH2* and *ADH3* loci. Alleles of *ADH2* found in whites and Asians are *ADH2*1* and *ADH2*2*, which encode for the low activity (β1) and high activity (β2) subunits, respectively. The kcat values for the resulting dimeric isozymes are very different: 9.2 min^{-1} for β1β1 and 400 min^{-1} for β2β2.[22] The *ADH2*2* frequency is much higher in Asians (60%-80%) than in whites (0%-10%).[21] *ADH3* alleles are *ADH3*1* and *ADH3*2*, which produce the γ1 and γ2 subunits. The γ1γ1 isozyme (kcat = 87 min^{-1}) is moderately more active than the γ2γ2 isozyme (kcat = 35 min^{-1}).[22] *ADH3*1* frequency is about 50% to 60% in whites and higher than 90% in Asians.[3,23]

A low prevalence of *ADH2*2*[4-18,20,24] and *ADH3*1*,[4,5,7,12,16-18] which encode for the highly active β2β2 and γ1γ1 isozymes, respectively, has been identified in alcoholic Asians, although some reports do not find differences in *ADH3* polymorphism between alcoholic and nonalcoholic Asians.[6,8] An explanation for both the *ALDH* and *ADH* allele distributions is that in each case, the enzymatic form resulting from the respective gene expression (*ALDH2*2*, *ADH2*2*, or *ADH3*1*) produces a higher concentration of acetaldehyde, either by a decreased oxidation rate to acetate or by a faster acetaldehyde production. A high acetaldehyde concentration results in uncomfortable symptoms that deter from excessive drinking. On the other hand, alcoholic individuals with the inactive *ALDH2*2* or the highly active *ADH2*2* or *ADH3*1* may be at increased risk for organ damage.[25] Several reports in Asians partially support this concept.[7,8,14,19,24,26,27]

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

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In Asians, the high frequency of the *ALDH2*2* genotype (10%-44%)[21] overshadows the effects of *ADH* variability. A study of the relationship between ADH polymorphism and alcohol-related diseases in Europeans would provide the opportunity for excluding the otherwise strong influence of ALDH deficiency. It would also be of interest to screen a population with personal and social attitudes toward alcohol that are different from those of the more studied Asian societies. However, the low prevalence of *ADH2*2* in whites[3] has precluded until now the finding of a significant correlation between this allele and alcohol pathology in Europe,[28-32] although a relationship similar to that found in Asians has been recently reported for Jewish[33] and Australian[34] men. Moreover, except for some alcohol-related cancers,[35-37] no correlation[28,30-32,38,39] or conclusive results[34,40,41] have been reported regarding the influence of the *ADH3* gene on alcohol-related pathologies in whites. In the present report we have analyzed a large European sample with the objective of determining the distribution of the ADH alleles in different European countries and correlating the ADH polymorphism with alcoholism and alcohol-induced cirrhosis in whites.

MATERIALS AND METHODS

Subjects. The study protocol was approved by the Ethical Committees of the 5 participating medical centers, and all patients gave written informed consent.

Genotyping was performed on blood from 876 white individuals, men and women, aged from 20 to 84 years, from the following populations: Tarragona (Spain), Bordeaux (France), Heidelberg (Germany), Stockholm (Sweden), and Kraków (Poland). Immigrants and their descendants were excluded. Subjects were classified into 4 groups according to their alcohol intake and the presence of liver disease. Men who consumed more than 100 g of pure alcohol per day and women with a daily alcohol intake of more than 70 g, for more than 10 years, were considered alcoholics (n = 425). They were all patients admitted to the hospital for alcohol detoxification and they fulfilled the diagnostic criteria for alcohol dependence.[42] Alcohol history was obtained by a face-to-face interview, and all these patients had a positive CAGE test. In doubtful cases, relatives were also interviewed. Alcoholics included patients with alcohol-induced cirrhosis and individuals with no liver disease. Patients with alcohol-induced pancreatitis were excluded. Nonalcoholic subjects (n = 451) were individuals who consumed less than 40 g (men) or 20 g (women) of pure alcohol per day, and did not meet the criteria for alcohol dependence.[42] They included healthy controls and patients with viral cirrhosis. Cirrhosis, either of viral or alcoholic origin was diagnosed, in most cases, by means of histopathologic examination of a liver sample obtained by percutaneous needle biopsy. In 7 subjects from Tarragona and 20 from Kraków, for whom biopsy was contraindicated (usually because of severe coagulation abnormalities), and in all patients from Stockholm, cirrhosis was established by clinical criteria: physical examination, liver function test, liver ultrasonography and, in most cases, demonstration of oesophageal varices through upper gastrointestinal endoscopy. Healthy controls were blood donors and hospital personnel with no history of alcoholism, no evidence of liver disease at physical examination, and normal liver function tests. Alcoholics with no liver disease met the alcoholism criteria mentioned above and had normal liver or minor nonspecific hepatic abnormalities at the above clinical analyses and, in some cases, at pathological examination of percutaneous needle biopsy of the liver. Biopsies in these individuals were performed for diagnostic purposes, usually because of the presence of an enlarged liver and/or abnormalities in liver function test (up to 2-fold the upper normal limit). Patients were excluded if they had serological evidence of previous hepatitis B, hepatitis C, or human immunodeficiency virus infection.

In 42 individuals (28 nonalcoholics and 7 alcoholics from Tarragona, and 7 alcoholics from Stockholm) only information regarding alcoholism was available, but data on the presence and type of liver disease were lacking or inconsistent. This subset of patients was included only in the calculations that related genotype and allele frequencies with alcoholism, but not in the calculations that related genotype and allele frequencies with liver disease.

Genotype Determination. Venous blood was blotted onto 3-mm filter paper (Whatman, Maidston, UK), dried, and stored at room temperature. The polymerase chain reaction was used to amplify polymorphic portions of exon 3 of the *ADH2* gene and of exon 8 of the *ADH3* gene[43] with specific primers.[7,44] Five-millimeter-diameter discs of the filter paper with the dried blood were placed directly in a 100 μL amplification mixture and overlaid with mineral oil. Genomic DNA was denatured by heating for 6 minutes at 96°C. *Thermus aquaticus* (*Taq*) DNA polymerase (Ecogen, Barcelona, Spain) (2.5 units) was added and used for 30 cycles of amplification (1 minute at 95°C, 45 seconds at 55°C, and 45 seconds at 72°C) in a thermal cycler (PTC-100, M.J. Research, Watertown, MA). For allele detection, aliquots of the amplified DNA products were digested with *Mae*III for *ADH2*2*, or with *Ssp*I (Roche Diagnostics, Mannheim, Germany) for *ADH3*1*. Products of digestion were run on 14% polyacrylamide gels and stained with ethidium bromide.

Statistical Analysis. Variation in allele frequencies between samples was analyzed using exact tests for population differentiation by means of the population genetics software GENEPOP.[45] Fisher's method[46] was used to obtain a single test of the aggregate populations after combining probabilities from tests of significance based on the independent samples. χ^2 goodness of fit tests were used to study agreement with Hardy-Weinberg expectations. Linkage disequilibria between *ADH2* and *ADH3* were estimated by means of the composite digenic disequilibrium coefficient Δ_{AB}.[47,48]

RESULTS

ADH2 Genotype. We compared allele frequencies of the distinct European populations using data only from healthy nonalcoholics (Table 1). The genotype distribution of all groups studied fit the expected Hardy-Weinberg equilibrium. The *ADH2*2* allele frequency ranged from 0% in Bordeaux to 5.4% in Tarragona, with an average of 2.2%. Exact tests for population differentiation did not detect statistically significant differences either when all populations were simultaneously compared ($P = .158$) or when all possible pairs of populations were contrasted with each other. Nonsignificant differences were also observed when groups of the same pathology were compared for each European population.

We have also compared the allele frequencies of healthy controls and patients with alcoholism and/or liver disease within each population. In no cases were differences found to be significant (results not shown).

The lack of differences between the healthy controls (Table 1) suggests that allele frequencies at *ADH2* are homogeneous across Europe (further supported by the analysis of the

TABLE 1. Genotype Number and Allele Frequencies (%) of *ADH2* in Healthy Controls of the Five European Populations Studied

		Genotype		Allele	
Population	n	*1/*1	*1/*2	*1	*2
Tarragona	37	33	4	94.6	5.4
Bordeaux	40	40	0	100	0
Heidelberg	41	40	1	98.8	1.2
Kraków	66	64	2	98.5	1.5
Stockholm	40	37	3	96.2	3.8
Total	224	214	10	97.8	2.2

NOTE. Differences between populations were not statistically significant.

TABLE 2. Genotype Number and Allele Frequencies (%) of *ADH2* in Europeans Grouped According to Alcoholism and/or Liver Disease

Group	n	Genotype		Allele	
		*1/*1	*1/*2	*1	*2
Healthy controls	224	214	10	97.8	2.2
Viral cirrhosis	199	184	15	96.2	3.8*†
Alcoholics with no liver disease	231	226	5	98.9	1.1*
Alcohol-induced cirrhosis	180	175	5	98.6	1.4†

*P = .009. Viral cirrhosis vs. alcoholics with no liver disease.
†P = .03. Viral cirrhosis vs. alcohol-induced cirrhosis.

nonalcoholics, see below), and therefore, all data could be combined in a single table (Table 2). Individuals were grouped according to their pathology, irrespective of country of origin, and the allele frequencies were compared. When the whole sample was considered, a lower prevalence of *ADH2**2 was observed in the groups of alcoholics with no liver disease and alcohol-induced cirrhosis. Differences were significant when these two groups were compared with the viral cirrhosis group (Table 2). The *ADH2**2 prevalence of the alcohol-induced cirrhosis group was similar to that of the alcoholics with no liver disease.

To test the influence of *ADH2* alleles on alcoholism, independently of the liver pathology, we compared all the nonalcoholic individuals (healthy controls and viral cirrhosis) with the alcoholic group (alcoholics with cirrhosis and alcoholics with no liver disease). For all populations but Bordeaux, the nonalcoholics exhibited a consistently higher *ADH2**2 frequency, statistically different (P = .0016) from that of the alcoholics after grouping all samples (Table 3). Although grouping is correct on statistical grounds, because no stratification is apparent, an overall test of the null hypothesis of no genotypic effects can also be accomplished combining significance values by Fisher's method.[46] In this case, statistical significance (P = .0490) is attained after excluding Bordeaux. Table 3 also shows a lack of difference in

TABLE 3. Genotype Number and Allele Frequencies (%) of *ADH2* in Alcoholic and Nonalcoholic Europeans

Group	Population	n	Genotype			Allele		P
			*1/*1	*1/*2	*2/*2	*1	*2	(NA vs. A)
NA	Tarragona	155	137	17	1	93.9	6.1	.1443
	Bordeaux	80	78	2	0	98.7	1.3	1
	Heidelberg	103	96	7	0	96.6	3.4	.0842
	Kraków	73	70	3	0	98.0	2.0	.3716
	Stockholm	40	37	3	0	96.2	3.8	.0921
	Total	451	418	32	1	96.2	3.8	.0016
A	Tarragona	96	90	6	0	96.9	3.1	
	Bordeaux	80	78	2	0	98.7	1.3	
	Heidelberg	81	80	1	0	99.4	0.6	
	Kraków	120	118	2	0	99.2	0.8	
	Stockholm	48	48	0	0	100	0	
	Total	425	414	11	0	98.7	1.3	

NOTE. Nonalcoholics (NA) include healthy controls and patients with viral cirrhosis. The alcoholic group (A) includes alcoholics with cirrhosis and without liver disease. Additional samples to those presented in Table 2, classified as nonalcoholics (n = 28) and alcoholics (n = 14), are included. Differences between populations within groups were not statistically significant. Combining probabilities (right column, excluding Bordeaux) from independent tests[46]: $-2\Sigma \ln P = 15.569$; $P_{(total)} = .049$.

TABLE 4. Genotype Number and Allele Frequencies (%) of *ADH2* in Europeans According to Gender and Drinking Habits

Group	n	Genotype			Allele	
		*1/*1	*1/*2	*2/*2	*1	*2
Men						
Nonalcoholics	239	217	21	1	95.2	4.8*
Alcoholics	288	280	8	0	98.6	1.4*
Women						
Nonalcoholics	212	201	11	0	97.4	2.6
Alcoholics	137	134	3	0	98.9	1.1

NOTE. Nonalcoholics and alcoholics are defined as in Table 3.
*P = .002 nonalcoholic men vs. alcoholic men.

ADH2 allele frequencies between European populations when nonalcoholics (healthy controls plus viral cirrhosis) are considered, reinforcing the conclusion on the homogeneity of the studied populations regarding the *ADH2* polymorphism, previously reached in the analysis of the healthy controls (Table 1).

The *ADH2* genotyping data are presented in Table 4 according to drinking habits and gender. In both men and women, the *ADH2**2 frequency was higher in nonalcoholics than in alcoholics, reaching statistical significance in men but not in women (P = .13).

ADH3 Genotype. Table 5 shows the *ADH3* gene frequencies found in the 5 European countries studied, considering only healthy controls. The genotype distribution of all groups studied fit the expected Hardy-Weinberg equilibrium. As for the *ADH2* gene, no differences were found in the allele distribution of *ADH3*. Therefore, the European population studied can be considered homogeneous also for the *ADH3* polymorphism.

Differences in *ADH3* allele distribution were also not significant when the control group was compared with the groups of patients with alcoholism and/or liver disease within each European population (data not shown). In Table 6 (4 top lines), data from all populations have been pooled and grouped according to the 4 defined categories. Also in this case, differences were not significant. Finally, individuals were grouped in alcoholics and nonalcoholics within each population, as previously performed for *ADH2* (Table 3), but in contrast with the *ADH2* analysis, no differences were found regarding the *ADH3* allele frequencies (not shown). When samples from all the study sites were combined, lack of differences was also observed between nonalcoholics and alcoholics (Table 6, 2 bottom lines), although genotype frequencies were different. Because results of Table 2 show that *ADH2* polymorphism correlates with alcoholism, we

TABLE 5. Genotype Number and Allele Frequencies (%) of *ADH3* in Healthy Controls of the Five European Populations Studied

Population	n	Genotype			Allele	
		*1/*1	*1/*2	*2/*2	*1	*2
Tarragona	37	7	25	5	52.7	43.3
Bordeaux	40	15	19	6	61.2	38.8
Heidelberg	41	9	21	11	47.6	52.4
Kraków	66	19	34	13	54.5	45.5
Stockholm	40	16	18	6	62.5	37.5
Total	224	66	117	41	55.6	44.4

NOTE. Differences between populations were not statistically significant.

TABLE 6. Genotype Number and Allele Frequencies (%) of *ADH3* in Europeans Grouped According to Alcoholism and/or Liver Disease

Group	n	Genotype			Allele	
		*1/*1	*1/*2	*2/*2	*1	*2
Healthy controls	224	66	117	41	55.6	44.4
Viral cirrhosis	199	56	114	29	57.2	42.8
Alcoholics with no liver disease	231	82	104	45	55.1	44.9
Alcoholic-induced cirrhosis	180	62	82	36	56.8	43.2
Totals						
Nonalcoholics	451	131*	246*	74*	56.3	43.7
Alcoholics	425	150	191	84	57.7	42.3

NOTE. Nonalcoholics and alcoholics are as in Table 3. Differences in allele frequencies between groups were not significant.

*P = .023. Distribution of genotype frequencies was different between nonalcoholics and alcoholics.

performed again the *ADH3* statistical analysis after discarding all samples with the *ADH2*2* genotype to avoid the influence of *ADH2* polymorphism. However, even in this case, differences in allele frequencies were not significant (result not shown). Lack of differences was also found when the comparison between groups was separately performed for men (n = 527) and women (n = 349) (not shown).

The fact that the *ADH2* and *ADH3* genes are contiguous in chromosome 4q21-23 of the human genome[49] led us to investigate a possible association between the alleles of each locus. The digenic disequilibrium for all individuals analyzed (N = 876, $\Delta_{AB} = -0.009$; P = .003), as well as for the nonalcoholic and for the alcoholic groups analyzed separately (Table 7), clearly indicates that the *ADH2*1* allele is strongly associated with *ADH3*2* whereas *ADH2*2* is associated with *ADH3*1*. This association is not caused by a statistical mixing of the 5 populations, unmasking a possible stratification, because the same trend was found in all of them, although statistical significance was only achieved for the Tarragona population where sample size was larger (data not shown).

DISCUSSION

We report here, for a large European sample, that *ADH2*2* frequency is higher in nonalcoholics than in alcoholics. The low prevalence of the *ADH2*2* allele in Europeans precludes finding significant differences when each population analyzed is considered separately, because of the relatively small size of the sample, either when considering only healthy controls (Table 1) or nonalcoholics (Table 3). It should be noticed, however, that in the latter case a clear tendency towards a higher *ADH2*2* frequency in nonalcoholics is present in 4 of the 5 populations studied (Table 3). It is reasonable to assume that the same tendency would be found for the Bordeaux population if a larger sample was analyzed. The homogeneity of the studied populations regarding *ADH2* polymorphism allows the grouping of the data from the 5 countries, considering a single European population. Then differences between groups are more clear (Table 2) and they become highly significant when we classify all samples into only two groups, alcoholic (n = 425) and nonalcoholic (n = 451) (Table 3). *ADH2*2* frequency for nonalcoholics is 2.9-fold that for alcoholic subjects. This result fully agrees with the well-proven higher *ADH2*2* frequency in nonalcoholics of Asian populations compared with alcoholics (about 1.3- to 2-fold),[4-18,20,24] and it represents a strong support to the hypothesis that the *ADH2*2* allele is a genetic factor that decreases the risk for alcoholism. Thus, despite profound differences in both the polymorphism of other genes of alcohol metabolism (*ALDH2* and *ADH3*) and in the social habits towards alcohol, whites are influenced similarly as Asians by *ADH2* polymorphism in regard to alcohol addiction. The recent reports of an association of the *ADH2*2* allele with a reduced ethanol consumption in Jewish men[33] and Australian men of European origin[34] support the concept that the effect of *ADH2* polymorphism on alcohol drinking behavior is general. However, it is obvious that only a relatively small number of Europeans will be protected against alcoholism by possessing *ADH2*2* because of the low frequency of individuals with genotypes containing this allele (4.5% in the control group, Table 1).

When, in addition to the alcohol drinking habit, the gender of the individual is also considered, the higher frequency of *ADH2*2* in nonalcoholics is consistently found in both men and women, although the differences are not significant in women. A similar result was reported in a study with Australians of European origin, and several possible causes were suggested, including metabolic differences between men and women, and sample selection.[34] In our case a strong tendency towards a lower *ADH2*2* frequency in alcoholics (1.1%) is observed in women, suggesting that the lack of significance is only caused by the smaller number in the female group (Table 4).

It is reasonable to assume that within alcoholics, individuals with the *ADH2*2* allele will be at higher risk of developing alcohol-related organ damage, because of the accumulation of the highly toxic acetaldehyde.[25] However, our results do not support this hypothesis in regard to alcohol-induced cirrhosis. This group exhibits a similar *ADH2*2* frequency as the group of alcoholics without liver disease. In Asians, with the exception of reports by one research group,[19,24,27] all other studies are consistent with our results and suggest a lack of relationship between *ADH2* polymorphism and alcohol-induced cirrhosis.[7,8,14]

The frequency of the *ADH2*2* allele in healthy Europeans is low, with only 2.2% in a sample of 224 individuals. This result is consistent with previous reports on *ADH2* genotyping in whites.[21] The *ADH2*2* frequency found by genotyping is in several cases lower than the frequencies estimated by phenotyping experiments[3] suggesting that the phenotype technique, based on gel electrophoresis and pH analysis, sometimes overestimated the *ADH2*2* prevalence.[29,31]

Comparison of *ADH2* frequencies between populations

TABLE 7. Classification of Alcoholic and Nonalcoholic Individuals (n = 876) According to the *ADH2* and *ADH3* Genotypes to Determine Linkage Disequilibrium Between the Two Loci

Group	*ADH3*			*ADH2*
	*1/*1	*1/*2	*2/*2	
Nonalcoholics	114	234	70	*1/*1
	16	12	4	*1/*2
	1	0	0	*2/*2
Alcoholics	141	190	83	*1/*1
	9	1	1	*1/*2
	0	0	0	*2/*2

NOTE. Groups are as in Table 3. The digenic disequilibrium coefficient (Δ_{AB})[48] is −0.0108 (P = .028) for nonalcoholics and −0.0074 (P = .031) for alcoholics.

from distinct European countries shows no statistical evidence towards a geographically heterogeneous distribution of the *ADH2* alleles. It can be noticed, however, that a strong difference exists between two close areas, Tarragona and Bordeaux. This difference is consistent with data of previous independent research[29,31,32,38,39,50] and, although not statistically significant with the present size of the sample (Tables 1 and 3), it deserves further investigation.

The distribution of the *ADH3* alleles is also homogeneous among the European populations, with global frequencies of 55.6% for *ADH3*1* and 44.4% for *ADH3*2*, in agreement with previous reports.[20,22,30,47] The allele frequencies are very similar for all groups: controls, patients with viral cirrhosis, alcoholics with cirrhosis, and alcoholics with no liver disease (Table 6). Therefore, *ADH3* does not appear to play a causative role in the development of alcohol-induced cirrhosis, which is in accordance with a previous study.[39] Moreover, the fact that the allele frequencies are practically identical between the alcoholic and nonalcoholic individuals (Table 6) suggests that the influence of *ADH3* polymorphism on predisposition to alcohol abuse is small, in agreement with most previous studies in Europeans.[28,30-32,38,39] Although this result seems to contradict the conclusions reached on Asians, this may not be the case when linkage disequilibrium between *ADH2* and *ADH3* is considered (see below).

An important metabolic effect of the polymorphism at the *ADH2* locus is expected for the large difference in activity between the isozymes encoded by *ADH2*.[21] However, no differences in the rate of alcohol elimination have been reported between individuals with the *ADH2*1* and *ADH2*2* alleles.[51,52] In short, the basis for a protective effect of *ADH2*2* against alcohol misuse ("the ADH effect"),[53] is still not clear.[20] An appealing hypothesis is a role of the extrahepatic ethanol metabolism because the *ADH2* gene is expressed in many organs other than liver, such as the blood vessels.[54-56] Thus, although the total extrahepatic metabolism is small when compared with the liver contribution, the local ethanol oxidation may significantly influence the normal function of these tissues. This could be a basis for unpleasant symptoms after ethanol intake in individuals with the most active *ADH2*2* allele. This would also explain the ethanol-induced cutaneous erythema in *ADH2*2* subjects.[57]

We have proven that the European population shows linkage disequilibrium between *ADH2* and *ADH3*. The two most active alleles *ADH2*2* and *ADH3*1* are associated, therefore the probability that both alleles are simultaneously found in an individual is higher than in the case of random allele segregation. Evidence for this allele linkage has been found also in Asian populations[4,20,58] suggesting a general occurrence. Our results on allele frequencies in whites and many reports in Asians indicate that *ADH2* polymorphism has a stronger influence than *ADH3* polymorphism on the levels of alcohol intake. The *ADH2*2* frequency is low in Europeans, therefore the effect of the allele linkage must be small on the *ADH3* allele distribution between alcoholics and nonalcoholics, although it may contribute to the *ADH3* genotype differences found between both groups in the present work (Table 6). In the Asians, with a high prevalence of *ADH2*2*, the effect of the linkage on the *ADH3* distribution should be stronger.[58] Thus, the association with *ADH2*2* could provide an explanation for the high frequency of *ADH3*1* reported in nonalcoholic Asians.[4,5,7,12,16-18] In fact, when the linkage was considered no relationship was found between *ADH3* and alcoholism in recent studies with a large number of Chinese individuals.[20,58] Therefore, the influence of *ADH3* on ethanol consumption appears to be minimal in both European and Asian populations.

In conclusion, our data indicate that individuals of geographically distant European countries exhibit similar allele frequencies of the alcohol dehydrogenase class I genes *ADH2* and *ADH3*. Polymorphism at *ADH3* has no effect on the propensity to alcoholism, whereas *ADH2*2* decreases the risk of excessive alcohol intake, an effect now shown in Europe. Finally, the most active alleles *ADH2*2* and *ADH3*1* are associated in Europeans, a fact that should be taken into account when allele frequencies are correlated to alcoholism and alcohol-related disease.

REFERENCES

1. Harada S, Agarwal DP, Goedde HW. Human aldehyde dehydrogenase: 3,4-dihydroxyphenylacetaldehyde metabolizing isoenzymes. In: Weiner H, Wermuth B, eds. Enzymology of Carbonyl Metabolism. New York: A.R. Liss, 1982;1:147-153.
2. Shibuya A, Yoshida A. Genotypes of alcohol-metabolizing enzymes in Japanese with alcohol liver diseases: a strong association of the usual Caucasian-type aldehyde dehydrogenase gene (*ALDH2*[1]) with the disease. Am J Hum Genet 1988;43:744-748.
3. Agarwal DP, Goedde HW. Alcohol metabolism, alcohol intolerance and alcoholism. Biochemical and pharmacological approaches. Berlin: Springer, 1990.
4. Thomasson HR, Edenberg HJ, Crabb DW, Mai X-L, Jerome RE, Li T-K, Wang S-P, et al. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. Am J Hum Genet 1991;48:677-681.
5. Thomasson HR, Crabb DW, Edenberg HJ, Li T-K. Alcohol and aldehyde dehydrogenase polymorphisms and alcoholism. Behav Gen 1993;23:131-136.
6. Thomasson HR, Crabb DW, Edenberg HJ, Li T-K, Hwu H-G, Chen CC, Yeh EK, et al. Low frequency of the *ADH2*2* allele among Atayal natives of Taiwan with alcohol use disorders. Alcohol Clin Exp Res 1994;18:640-643.
7. Chao Y-C, Liou S-R, Chung Y-Y, Tang H-S, Hsu C-T, Li T-K, Yin S-J. Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. HEPATOLOGY 1994;19:360-366.
8. Chao Y-C, Young TH, Tang H-S, Hsu CT. Alcoholism and alcoholic organ damage and genetic polymorphisms of alcohol metabolizing enzymes in Chinese patients. HEPATOLOGY 1997;25:112-117.
9. Muramatsu T, Zu-Cheng W, Yi-Ru F, Kou-Bao H, Heqin Y, Yamada K, Higuchi S, et al. Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shangai. Hum Genet 1995;96:151-154.
10. Maezawa Y, Yamauchi M, Toda G, Suzuki H, Sakurai S. Alcohol-metabolizing enzyme polymorphisms and alcoholism in Japan. Alcohol Clin Exp Res 1995;19:951-954.
11. Higuchi S, Matsushita S, Murayama M, Takagi S, Hayashida M. Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. Am J Psychiat 1995;152:1219-1221.
12. Higuchi S, Muramatsu T, Matsushita S, Murayama M, Hayashida M. Polymorphisms of ethanol-oxidizing enzymes in alcoholics with inactive ALDH2. Hum Genet 1996;97:431-434.
13. Higuchi S, Matsushita S, Muramatsu T, Murayama M, Hayashida M. Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. Alcohol Clin Exp Res 1996;20:493-497.
14. Tanaka F, Shiratori Y, Yokosuka O, Imazeki F, Tsukada Y, Omata M. High incidence of *ADH2*1/ALDH2*1* gene among Japanese alcohol dependents and patients with alcoholic liver disease. HEPATOLOGY 1996;23:234-239.
15. Tanaka F, Shiratori Y, Yokosuka O, Imazeki F, Tsukada Y, Omata M. Polymorphism of alcohol-metabolizing genes affects drinking behavior and alcoholic liver disease in Japanese men. Alcohol Clin Exp Res 1997;21:596-601.
16. Nakamura K, Iwahashi K, Matsuo Y, Miyatake R, Ichikawa Y, Suwaki H. Characteristics of Japanese alcoholics with the atypical aldehyde dehydrogenase 2*2. I. A comparison of the genotypes of *ALDH2*, *ADH2*, *ADH3*, and cytochrome P-4502E1 between alcoholics and nonalcoholics. Alcohol Clin Exp Res 1996;20:52-55.
17. Chen WJ, Loh EW, Hsu Y-PP, Chen C-C, Yu J-M, Cheng ATA.

Alcohol-metabolizing genes and alcoholism among Taiwanese Han men: independent effect of *ADH2*, *ADH3* and *ALDH2*. Br J Psychiatry 1996;168:762-767.
18. Shen YC, Fan JH, Edenberg HJ, Li T-K, Cui Y-H, Wang Y-F, et al. Polymorphism of *ADH* and *ALDH* genes among four ethnic groups in China and effects upon the risk for alcoholism. Alcohol Clin Exp Res 1997;21:1272-1277.
19. Yamauchi M, Maezawa Y, Mizuhara Ohata M, Hirakawa J, Nakajima H, Toda G. Polymorphisms in alcohol metabolizing enzyme genes and alcoholic cirrhosis in Japanese patients: a multivariate analysis. HEPATOLOGY 1995;22:1136-1142.
20. Chen C-C, Lu R-B, Chen Y-C, Wang M-F, Chang Y-C, Li T-K, Yin S-J. Interaction between the functional polymorphisms of the alcohol metabolism genes in protection against alcoholism. Am J Hum Genet 1999;65:795-807.
21. Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, Bhatia K, et al. Distribution of *ADH2* and *ALDH2* genotypes in different populations. Hum Genet 1992;88:344-346.
22. Bosron WF, Li T-K. Catalytic properties of human liver alcohol dehydrogenase isoenzymes. Enzyme 1987;37:19-28.
23. Bosron WF, Li T-K. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenase, and their relationship to alcohol metabolism and alcoholism. HEPATOLOGY 1986;6:502-510.
24. Yamauchi M, Maezawa Y, Toda G, Suzuki H, Sakurai S. Association of a restriction fragment length polymorphism in the alcohol dehydrogenase 2 gene with Japanese alcoholic liver cirrhosis. J Hepatol 1995b;23:519-523.
25. Couzigou P, Coutelle C, Fleury B, Iron A. Alcohol and aldehyde dehydrogenase genotypes, alcoholism and alcohol related disease. Alcohol Alcohol 1994;2:21-27.
26. Matsumoto M, Takahashi H, Maruyama K, Higuchi S, Matsushita S, Muramatsu T, Okuyama K, et al. Genotypes of alcohol-metabolizing enzymes and the risk for alcoholic chronic pancreatitis in Japanese alcoholics. Alcohol Clin Exp Res 1996;20:289-292.
27. Yamauchi M. Association of polymorphism in the alcohol dehydrogense 2 gene with alcohol-related organ injuries, especially liver cirrhosis. Addict Biol 1998;3:151-157.
28. Ricciardi BR, Saunders JB, Williams R, Hopkinson DA. Hepatic ADH and ALDH isoenzymes in different racial groups and in alcoholism. Pharmacol Biochem Behav 1983;18:61-65.
29. Vidal F, Pérez J, Panisello J, Toda R, Gutiérrez C, Richart C. Atypical liver alcohol dehydrogenase in Spanish population. Its relation with the development of alcoholic liver disease. Alcohol Clin Exp Res 1993;17: 782-785.
30. Gilder FJ, Hodgkinson S, Murray RM. ADH and ALDH genotype profiles in Caucasians with alcohol-related problems and controls. Addiction 1993;88:383-388.
31. Parés X, Farrés J, Parés A, Soler X, Panés J, Ferré JL, Caballería J, et al. Genetic polymorphism of liver alcohol dehydrogenase in Spanish subjects: significance of alcohol consumption and liver disease. Alcohol Alcohol 1994;29:701-705.
32. Espinós C, Sánchez F, Ramírez C, Juan F, Nájera C. Polymorphism of alcohol dehydrogenase genes in alcoholic and non-alcoholic individuals from Valencia (Spain). Hereditas 1997;126: 247-253.
33. Neumark YD, Friedlander Y, Thomasson HR, Li T-K. Association of the ADH2*2 allele with reduced ethanol consumption in Jewish men in Israel: a pilot study. J Stud Alcohol 1998;59:133-139.
34. Whitfield JB, Nightingale BN, Bucholz KK, Madden PAF, Heath AC, Martin NG. ADH genotypes and alcohol use and dependence in Europeans. Alcohol Clin Exp Res 1998;22:1463-1469.
35. Coutelle C, Ward PJ, Fleury B, Quattrocchi P, Chambrin H, Iron A, Couzigou P, et al. Laryngeal and oropharyngeal cancer, and alcohol dehydrogenase 3 and glutathione S-transferase M1 polymorphisms. Hum Genet 1997;99:319-325.
36. Harty LC, Caporaso NE, Hayes RB, Winn DM, Bravo-Otero E, Blot WJ, Kleinman DV, et al. Alcohol dehydrogenase 3 genotype and risk of oral cavity and pharyngeal cancers. J Nat Can Ins 1997;22:1698-1705.
37. Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Graham, S, Marshall JR, Muti P, et al. Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk. Can. Causes Control 1999;10:369-377.
38. Couzigou P, Fleury B, Groppi A, Cassaigne A, Begueret J, Iron A. Genotyping study of alcohol dehydrogenase class I polymorphism in French patients with alcoholic cirrhosis. Alcohol Alcohol 1990;25:623-626.
39. Poupon RE, Nalpas B, Coutelle C, Fleury B, Couzigou P, Higueret D, The French Group for Research on Alcohol and Liver. Polymorphism of alcohol dehydrogenase, alcohol and aldehyde dehydrogenase activities: implication in alcoholic cirrhosis in white patients. HEPATOLOGY 1992;15: 1017-1022.
40. Day CP, Bashir R, James OFW, Bassendine MJ, Crabb DW, Thomasson HR, Li T-K, et al. Investigation of the role of polymorphisms at the alcohol and aldehyde dehydrogenase loci in genetic predisposition to alcohol-related end-organ damage. HEPATOLOGY 1991;14:798-801.
41. Poupon RE, Ward P, Balkau B. Alcohol dehydrogenase polymorphisms and predisposition to alcoholic cirrhosis. HEPATOLOGY 1993;18:231-232.
42. Grant BF, Hartfort TC, Hasin DS, Chou P, Pickering R. DMS-III-R and the proposed DMS-IV alcohol use disorders. United States 1989: a nosological comparison. Alcohol Clin Exp Res 1992;16:215-221.
43. Xu Y, Carr LG, Bosron WF, Li T-K, Edenberg HJ. Genotyping of human alcohol dehydrogenases at the *ADH2* and *ADH3* loci following DNA sequence amplification. Genomics 1988;2:209-214.
44. Groppi A, Begueret J, Iron A. Improved methods for genotype determination of human alcohol dehydrogenase (ADH) at *ADH2* and *ADH3* loci using polymerase chain reaction-directed mutagenesis. Clin Chem 1990;36:1765-1768.
45. Raymond M, Rousset F, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 86:248-249.
46. Fisher RA. Statistical Methods for Research Workers, 9th ed. Edinburgh: Oliver and Boyd, 1944.
47. Weir BS, Cockerham CC. Complete characterization of disequilibrium at two loci. In: Feldman ME, ed. Mathematical Evolutionary Theory. Princeton: Princeton Univ. Press, 1989;86-110.
48. Weir BS. Genetic data analysis. Sunderland, MA: Sinauer, 1990.
49. Yasunami M, Kikuchi I, Sarapata D, Yoshida A. The human class I alcohol dehydrogenase gene cluster: three genes are tandemly organized in an 80-kb-long segment of the genome. Genomics 1990;7:152-158.
50. Coutelle C, Ward PJ, Quattrocchi P, Fleury B, The French Group for Research on Alcohol and Liver. Population distribution of alcohol dehydrogenase class I in France: comparison with other populations, and distribution with respect to gender and age. Alcohol Alcohol 1998;33:173-183.
51. Yamamoto K, Ueno Y, Mizoi Y, Tatsuno Y. Genetic polymorphism of alcohol and aldehyde dehydrogenase and the effects on alcohol metabolism. Jpn J Alcohol Drug Depend 1993;28:13-25.
52. Mizoi Y, Yamamoto K, Ueno Y, Fukunaga T, Harada S. Involvement of genetic polymorphisms of alcohol and aldehyde dehydrogenases in individual variation of alcohol metabolism. Alcohol Alcohol 1994;29:707-710.
53. Whitfield JB. Meta-analysis of the effects of alcohol dehydrogenase genotype on alcohol dependence and alcoholic liver disease. Alcohol Alcohol 1997;32:613-619.
54. Estonius M, Svensson S, Höög J-O. Alcohol dehydrogenase in human tissues: localisation of transcripts coding for five classes of the enzyme. FEBS Lett 1996;397:338-342.
55. Allali-Hassani A, Martínez SE, Peralba JM, Vaglenova J, Vidal F, Richart C, Farrés J, et al. Alcohol dehydrogenase of human and rat blood vessels. FEBS Lett 1997;405:26-30.
56. Goedde HW, Harada S, Agarwal DP. Alcohol metabolizing enzymes: studies of isozymes in human biopsies and cultured fibroblasts. Clin Genet 1979;16:29-33.
57. Takeshita T, Mao X-Q, Morimoto K. The contribution of polymorphism in the alcohol dehydrogenase β subunit to alcohol sensitivity in a Japanese population. Hum Genet 1996;97:409-413.
58. Osier M, Patstis AJ, Kidd JR, Lee J-F, Yin S-J, Ko H-C, Edenberg HJ, et al. Linkage disequilibrium at the *ADH2* and *ADH3* loci and risk of alcoholism. Am J Hum Genet 1999;64:1147-1157.