Genetic polymorphisms of ADH₂, ADH₃, CYP₄₅₀₂E₁ Dra-I and Pst-I, and ALDH₂ in Spanish men: lack of association with alcoholism and alcoholic liver disease

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Background/Aims: The relationship between polymorphisms at the alcohol dehydrogenase 2 (ADH₂), ADH₃, CYP₄₅₀₂E₁ and aldehyde dehydrogenase 2 (ALDH₂) loci and the individual predisposition to alcoholism and alcoholic liver disease in Caucasians is controversial.

Methods: We determined the genotypes of ADH₂, ADH₃, CYP₄₅₀₂E₁ (Pst-I and Dra-I) and ALDH₂ in 519 male Spaniards: 264 alcoholic subjects (47 without liver disease, 118 with non-cirrhotic liver disease and 99 with cirrhosis) and 255 non-alcoholic subjects (64 healthy controls, 110 with non-cirrhotic non-alcoholic liver disease and 81 with cirrhosis unrelated to alcohol). Genotyping was performed using PCR-RFLP methods on white cell DNA.

Results: The distribution of the allelic variants (allele *1 and allele *2) in the whole subjects analyzed was: ADH₂ 93.1% and 6.9%; ADH₃ 55.7 and 44.3%; CYP₄₅₀₂E₁ Dra-I 11.2 and 88.8%; CYP₄₅₀₂E₁ Pst-I 96.2 and 3.8% and ALDH₂ 100 and 0%, respectively. No differences were observed in the allelic distributions of the alcoholic and non-alcoholic subjects for the loci examined. Allele distribution in alcoholics with no liver disease, with alcoholic steatosis or hepatitis, and with cirrhosis unrelated to alcohol was also similar.

Conclusions: ADH₂, ADH₃, and CYP₄₅₀₂E₁ Pst-I and Dra-I genetic variations are not related to alcoholism or susceptibility to alcoholic liver disease in our male population. ALDH₂ locus is monomorphic.

Keywords: Alcohol; Alcohol dehydrogenase; Aldehyde dehydrogenase; Cytochrome P₄₅₀₂E₁; Alcoholic liver disease; Cirrhosis

1. Introduction

Alcoholism and alcohol-induced liver damage are clinically heterogeneous diseases that result from a likely multiplicity of interactive genetic and environmental influences, rather than a major single gene effect [1–5]. In order to study this genetic approach, several genes must be analyzed in a susceptible population. Several ‘candidate’ genes have been proposed and studied in recent years [6–12] and, among them, genes encoding ethanol and acetaldehyde-metabolizing enzymes such as alcohol dehydrogenase (ADH), cytochrome P₄₅₀₂E₁ (CYP₄₅₀₂E₁), and aldehyde dehydrogenase (ALDH) have been extensively studied, often with controversial or non-conclusive results, especially in whites [6,7,10,13,15].

ALDH₂ is the most important alcohol-metabolizing gene that affects predisposition to alcoholism and alcoholic liver disease in Asian populations. The ALDH₂*2 allele, which encodes for an inactive ALDH form, appears to protect against alcoholism [4,7,10]. Furthermore, alcoholics with this inactive allele may be at a greater risk of advanced
alcoholic liver disease [15–21]. However, the ALDH2*2 allele has not been found in Caucasians [10–22].

ADH presents genetic variability at the ADH2 and ADH3 loci. Two alleles in both loci (*1 and *2) have been described [23]. It has been reported that the prevalence of the more active alleles ADH2*2 [13,14,16–21,24,25] and ADH3*1 [13–15,19] is low in alcoholic Asians. Likewise, alcoholics with the highly active ADH2*2 or ADH3*1 may be at increased risk of organ damage [26], as has been shown in Asians [15,18,25,27]. A similar relationship has been reported for Jewish and Australian men [28,29]. Studies regarding ADH3*1 in whites are more controversial, showing no correlation [30–36], protection against alcoholism [37], or non-conclusive results [22,29,38].

CYPs2E1 is responsible for 10% of total ethanol metabolism, but it can be induced by chronic alcohol administration [39]. The CYPs2E1 gene also exhibits polymorphism. Two point mutations in the 5’ flanking region of the gene (Pst-I, Rsa-I) are in close linkage disequilibrium and alter the transcriptional activity of the gene [40]. So far, studies have failed to demonstrate that this polymorphism is related to an increased risk of alcohol dependence [41]. An association with alcoholic liver disease has been documented in the Japanese [18], but only one report has been able to find a similar association in Caucasians [42]. Less information is available regarding other mutations that affect this gene (Dra-I, Msp-I) [41,43].

In the present work, we have studied the frequency of ADH2, ADH3, ALDH2, and CYPs2E1 Dra-I and Pst-I polymorphisms and their relation to alcoholism and alcoholic liver disease (ALD) in 519 subjects living in Tarragona (Catalonia, Spain), a region with a long tradition of producing and consuming alcoholic beverages, specially wine.

2. Material and methods

2.1. Subjects

We studied 519 Spanish white men between 20 and 84 years of age at the Hospital Universitari de Tarragona Joan XXIII (Spain). Immigrants from other countries and their descendants were excluded. Subjects were classified into two groups according to their alcohol intake: alcoholics and non-alcoholics. Each group was divided further into the following subgroups: controls, non-cirrhotic liver disease, and cirrhosis of the liver.

People who drank a total amount of alcohol in the beverages ingested greater than 100 g/day for more than 10 years were considered alcoholics. The drinking history was obtained by a face-to-face interview based on a standardized questionnaire. In doubtful cases, relatives were also interviewed. Ninety-six percent of alcoholics met the DSM-IV diagnostic criteria for alcoholism [44].

Alcoholic subjects included patients with alcohol-induced cirrhosis, non-cirrhotic alcoholic liver disease (hepatitis and/or steatosis) and heavy drinkers without liver disease. The different types of ALD were diagnosed by examination of a liver biopsy. Alcoholics with no liver disease were diagnosed by means of percutaneous needle biopsy of the liver, usually done because of the presence of an enlarged liver and/or abnormalities in the liver enzyme levels, or during elective abdominal surgery. Histologic examination of these samples indicated a normal liver.

Non-alcoholic subjects were people who drank less than 10 g/day of alcohol. They included healthy controls and patients with non-alcoholic chronic liver disease (in most cases chronic hepatitis or cirrhosis due to HCV infection, diagnosed by liver biopsy examination). Healthy controls were people with no history of alcoholism or chronic disease, no evidence of liver disease at physical examination, and normal liver function tests. Information about toxic and medical history was collected through a short standardized questionnaire.

The study protocol was approved by the Ethical Committee of our hospital. Informed consent was obtained from each subject.

2.2. Blood samples

A 10 ml sample of blood was drawn in an EDTA vacutainer by venipuncture. Within 1 h of drawing, buffy coat was separated from the blood by centrifugation at 800 g for 10 min. Genomic DNA was isolated from the buffy coat using QiAAMP spin columns (Qiagen, Chatsworth, CA).

2.3. Analytical methods: genotype determination

Restriction fragment length polymorphisms (RFLP) in the ADH2, ADH3, CYPs2E1, and ALDH2 genes were detected by digesting PCR-amplified DNA [19,40,41]. For each PCR analysis, 100 ng of DNA was used. PCR analyses were performed with a Perkin Elmer 9700 Thermal Cycler. RFLP were detected by ethidium bromide staining after agarose gel electrophoresis.

2.3.1. ADH2 and ADH3 genotypes

The amplification reactions were carried out in a final volume of 15 μl containing 1.5 mM of MgCl2, 0.2 mM of each nucleotide (Boehringer Mannheim, Germany), 0.2 μM of each primer and 2 units of Thermus aquaticus (Taq) DNA polymerase (Gibco BRL). DNA was amplified for 35 cycles. Each cycle consisted of 1 min denaturation at 94 °C, 45 s annealing at 55 °C and 5 min extension at 72 °C. The primers used were: 5’ATCTTTTCTGATCTGAAAC3’ and 5’GAAGGGGGTCCACGGTG3’ for ADH2 genotypes, and 5’GCTTTAAGAGTAAATACTGTTCCCS3’ and 5’AAATCTACCTCTTCTCCAGACG3’ for ADH3 genotypes (Gibco BRL). For allele detection, aliquots of the amplified DNA products were digested with MaelII at 55 °C for ADH2, or with SspI at 37 °C (Roche Molecular Biochemicals) for ADH3. Digestion products were run on 2.5% high resolution agarose gels and stained with ethidium bromide. The genotypes identified were named according to the presence or absence of the enzyme restriction sites. So MaelII G/G = *1/*1, G/A = *1/*2 and A/A = *2/*2 are homozygotes for the absence of site (95 bp), heterozygotes (60/35/95 bp), and homozygotes for the presence of site (60/35 bp). SspI G/G = *1/*1, G/A = *1/*2 and A/A = *2/*2 are homozygotes for the absence of site (130 pb), heterozygotes (67/63/130 bp), and homozygotes for the presence of site (67/63 bp).

2.3.2. CYPs2E1 genotypes

The amplification reactions were conducted in a final volume of 50 μl containing 1.5 mM of MgCl2, 0.2 mM of each nucleotide (Boehringer Mannheim, Germany), 0.2 μM of each primer and 2 units of Thermus aquaticus (Taq) DNA polymerase (Gibo BRL). DNA was amplified for 35 cycles. Each cycle consisted of 30 s denaturation at 95 °C, 30 s annealing at 54 °C for the Pst-I polymorphism and at 63 °C for the Dra-I polymorphism, and 45 s extension at 72 °C. The primers used were: 5’ATCCTTCTGCTCTCTACAGTG3’ and 5’CCAGTCGAGTCTACATTGTCA3’ for ADH2, or with 5’AGTCGAGTGATGGATCC3’ and 5’GACAGGGTTTCATCATGTGG3’ for Dra-I (Gibo BRL). For allele detection, aliquots of the amplified DNA products were digested with Pst-I, Dra-I and MaelII at 37 °C (Roche Molecular Biochemicals). Digestion products were run on 4% agarose gels and stained with ethidium bromide. The genotypes identified were named according to the presence or absence of the enzyme restriction sites. So MaelII G/G = *1/*1, G/A = *1/*2 and A/A = *2/*2 are homozygotes for the absence of site (290/120 bp), heterozygotes (249/126/375 bp), and homozygotes for the presence of site (290/120 bp). Dra-I *1/*1, *1/*2 and 2*/*2 are homozygotes for the absence of site (375 bp), heterozygotes (249/126/375 bp), and homozygotes for the presence of site (249/126 bp).
3.2. ADH2 gene polymorphisms

The allelic distribution of the ADH2*1 and ADH2*2 genotypes in the whole population analyzed was 93.1 and 6.9%, respectively (Table 2). We compared the allelic frequencies observed in the different groups defined (alcoholics vs. non-alcoholics, controls vs. liver disease patients), and, in no cases were the differences found to be significant (Table 3).

3.3. ADH3 gene polymorphisms

The allele distribution in the whole series was 55.7% for ADH3*1 and 44.3% for ADH3*2. Table 4 shows the ADH3 allele distribution according to alcoholism and/or liver disease. Differences were not significant when the control group was compared with the different groups of patients with alcoholism and/or liver disease. Likewise, when individuals were put in groups of alcoholics and non-alcoholics, as had previously been done for ADH2, no differences were found regarding the ADH3 allele frequencies (Table 4).

3.4. Linkage disequilibrium between ADH2 and ADH3 loci

An association in the direction ADH2*2–ADH3*1 was observed, but no significant linkage disequilibrium could be demonstrated. The digenic disequilibrium coefficients were \( \Delta_{AB} = -0.0143, P = 0.21 \), for the non-alcoholic group and \( \Delta_{AB} = -0.0064, P = 0.45 \), for the alcoholics.

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Table 2

Genotype number and allele frequencies (%) of ADH2, ADH3, CYP2E1 Dra-I and Pst-I, and ALDH2 in the series analyzed.
3.5. CYP4502E1 gene polymorphisms in the Dra-I locus

The overall allele frequencies for the rare d1 and common d2 alleles in the Dra-I locus were 11.2 and 88.8%, respectively.

The rare d1 allele was slightly more common among subjects with alcohol-related cirrhosis than among heavy drinkers without alcohol-related liver disease, but the differences were not significant. The mutation was less frequent in the non-alcoholic cirrhosis group (8%), but it was not statistically significant (Table 5). The distribution of the CYP4502E1 genotypes for the Dra-I rare d1 allele was also similar in alcoholics and non-alcoholics (Table 5).

3.6. CYP4502E1 gene polymorphisms in the Pst-I locus

The global allelic frequencies for the common c1 and rare c2 alleles in the Pst-I locus were 96.2 and 3.8%, respectively (Table 2). The results of allelic and genotypic distributions observed in the groups defined are shown in Table 6.

3.7. Analysis of ADH-CYP4502E1 polymorphism associations

No evidence of gene–gene interaction was observed in relation to alcohol consumption or the development of ALD, when the polymorphic ADH and CYP4502E1 systems were analyzed together.

3.8. ALDH2 gene polymorphisms

We evaluated these polymorphisms in 100 individuals, 50 alcoholics and 50 non-alcoholics, either with and without liver disease. All subjects expressed the 1/1 genotype (Table 2).
4. Discussion

The results of the present work indicate, for a large and homogeneous Spanish male sample, that ADH2, ADH3 and CYP4502E1 Dra-I and Pst-I genotypes are not related to the individual risk of alcoholism or the development of advanced alcoholic liver disease. We have not detected polymorphism at the ALDH2 locus.

The highly active ADH2*2 allele is very frequent in Asians (60–80%) but not in whites (0–10%) [10]. Our results showed an ADH2*2 allele frequency of 7.8% for our healthy Spanish controls, higher than the one reported in French [33], Americans [48], Germans, Swedes and Finns [10,49], but lower than in Turks [10] and Swiss [50]. It is similar to frequencies described in other Spanish samples [51,52]. It is difficult to find an explanation for the higher ADH2*2 frequency in our population, but perhaps reflects migration patterns and the occupation of Spain historically by eastern populations.

It has been reported in Asians that the risk of alcohol dependence and alcoholic liver disease associated with ADH2*1 is greater than the risk associated with ADH2*2 [13,14,16,17,19–21,24,25]. Our results suggest that there is no relationship between the atypical ADH2 and alcohol abuse in Spaniards, because the frequencies of ADH2*2 obtained in alcoholics and non-alcoholics are very similar (7.4% vs. 6.5%, respectively). These results are in agreement with most previous studies in whites [48,52]. Only two reports in Jewish from Israel [28] and in Australian whites [29] have found a relationship similar to that found in Asians.

The two ADH2 alleles encode for dimeric isoenzymes with different metabolic ratios of ethanol to acetaldehyde. The ADH2*2 encodes a very active enzyme and may be expected to generate more acetaldehyde because of the higher activity. So, it could be expected that alcoholics with the more active b5b2 isoenzyme were at greater risk of ethanol intake causing tissular damage due to an increased accumulation of acetaldehyde. However, our results do not confirm this hypothesis for the development of alcoholic cirrhosis, because the allelic frequencies observed are similar for alcoholics, either with or without liver disease. These results agree with other results in Caucasians and Asians [30,31]. Only one research group has reported that alcoholics expressing the allele *2 are at greater risk [18, 25,27]. Moreover, the high frequency of the ALDH2*2 allele in Asians overshadows the effects of ADH variability. This strong influence could be provided in Europeans, since the ALDH2*2 is virtually absent in Caucasians, as demonstrated in the present work.

Regarding ADH3, important differences are also observed in allelic distribution between Asian and white populations: ADH3*1 is more prevalent in Asians (>90%) than in whites (50–60%) [12,48]. Our genotype distribution is consistent with previous reports in whites [21,23, 34,37,46].

Several reports in Asians have suggested that alcoholics with the more active ADH3*1 may be at greater risk for developing alcoholic liver disease [15,18]. This correlation has not been proved in whites [22,29–38]. The allelic frequencies are very similar for all the groups studied, and among alcoholics and non-alcoholics. So, in our population, ADH3 variation does not play a causative role in the predisposition to alcoholism or ALD.

The fact that class I ADH genes all lie within 80 kb on chromosome 4 led to the hypothesis that variants were not inherited independently. ADH2 and ADH3 genes are contiguous in the region 4q21-23 [53], and evidence of allele linkage has been found in Asians [13,21,54]. Recently, this linkage has also been reported in Europeans [37]. Our results show that the ADH2*2 and ADH3*1 alleles are associated, but they do not demonstrate a significant disequilibrium linkage in our population. However, the low ADH2*2 frequency in Caucasians means that the effect of the allele linkage on the ADH3 distribution must be smaller than in Asians. In Oriental populations, the excess of ADH3*1 observed in non-alcoholics could be influenced by the association with ADH2*2 [13,15–17].

Polymorphism of CYP4502E1 has been shown to influence the risk for ALD in some reports [43,55], but others have failed to find such an association [56]. Additionally, to date, no evidence of influence on alcoholism and alcohol dependence has been reported [41]. In the population analyzed, the allelic distribution for the CYP4502E1 Dra-I polymorphism among alcoholic and non-alcoholic subjects did not show differences. The frequencies of the rare Dra-I d1 allele were comparable in non-alcoholic population and alcoholics, and similar to those reported for Caucasians by other authors [31,41,43]. Nevertheless, we were unable to confirm the lower frequency of the d1 allele in alcoholics with liver cirrhosis commented on in some reports [43].

Regarding the CYP4502E1 Pst-I polymorphism, we found no association between the c2 mutation in the 5'-flanking region of the gene and a higher risk of alcoholism, since the mutant allele was detected in a comparable percent of alcoholic and non-alcoholic population. A relationship with alcoholic cirrhosis has been suggested in Asians, but results are controversial [16,57]. Although rare in Caucasians, this allele has been found to increase the risk of advanced alcoholic liver disease, particularly in patients with the less active isoenzymes of ADH3 [42]. Our results do not confirm this but demonstrated that the c2 allele is less frequent in alcoholics with liver cirrhosis than in alcoholics without cirrhosis or advanced liver disease. The combination study between the CYP4502E1/ADH3 genotypes and risk of alcohol-related liver disease was also negative. CYP4502E1 variants were not seen to be associated with alcoholism or risk of alcoholic liver disease, perhaps because of their low prevalence, which may explain why different reports have come up with different results.
We can conclude that polymorphisms of ADH2, ADH3, and CYP4502E1 are not related to the risk for developing alcoholism and/or alcoholic liver disease, at least in a large Caucasian population such as the one presented here, and that the allele ALDH2*2 is not expressed in the population analyzed.

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