Genetic Polymorphisms of Genes Coding to Alcohol-Metabolizing Enzymes in Western Mexicans: Association of *CYP2E1*c2/CYP2E1*5B* Allele with Cirrhosis and Liver Function

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Background: Alcoholic cirrhosis constitutes a major public health problem in the world where *ADH1B*, *ALDH2*, and *CYP2E1* polymorphisms could be playing an important role. We determined *ADH1B*2*, *ALDH2*2*, and *CYP2E1*c2* allele frequencies in healthy control individuals (C) and patients with alcoholic cirrhosis (AC) from western Mexico.

Methods: Ninety C and 41 patients with AC were studied. Genotype and allele frequency were determined through polymerase chain reaction-restriction fragment length polymorphisms.

Results: Polymorphic allele distribution in AC was 1.6% *ADH1B*2*, 0.0% *ALDH2*2*, and 19.5% *CYP2E1*c2;* in C: 6.1% *ADH1B*2*, 0% *ALDH2*2*, and 10.6% *CYP2E1*c2*. *CYP2E1*c2* polymorphic allele and *c1/c2* genotype frequency were significantly higher ($p < 0.05$ and $p < 0.01$, respectively) in patients with AC when compared to C. Patients with AC, carrying the *CYP2E1*c2* allele, exhibited more decompensated liver functioning evaluated by total bilirubin and prothrombin time, than *c1* allele carrying patients ($p < 0.05$). Cirrhosis severity, assessed by Child's Pugh score and mortality, was higher in patients carrying the *c2* allele, although not statistically significant.

Conclusions: In this study, *CYP2E1*c2* allele was associated with susceptibility to AC; meanwhile, *ADH1B*2* and *ALDH2*2* alleles were not. *CYP2E1*c2* allele was associated with AC severity, which could probably be attributed to the oxidative stress promoted by this polymorphic form. Further studies to clearly establish *CYP2E1*c2* clinical relevance in the development of alcohol-induced liver damage and its usefulness as a probable prognostic marker, should be performed. Also, increasing the number of patients and including a control group conformed by alcoholic patients free of liver damage may render more conclusive results. These findings contribute to the understanding of the influence of gene variations in AC development among populations, alcohol metabolism, and pharmacogenetics.

Key Words: Alcoholic Liver Disease, Polymorphisms, Pharmacogenetics, Oxidative Stress, Obesity.

CIRRHOSIS CONSTITUTES A major multifactorial public health problem in the world, where individual susceptibility attributed to alcohol abuse and genetic variability, among other factors, play an important role (Douds et al., 2003). Main hepatic ethanol metabolism involves alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2), producing acetaldehyde and acetate, respectively. When alcohol consumption becomes a chronic condition, cytochrome P4502E1 (CYP2E1) activates catalyzing ethanol conversion to acetaldehyde because it

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corresponds to the alcohol-induced microsomal system (Gemma et al., 2006; Lieber, 2000, 2004a,b). Accumulation of acetaldehyde and oxygen reactive species, considered responsible of liver injury, promoted by some genetic polymorphisms of the ethanol metabolic pathway, has been described (Dey and Cederbaum, 2006; Rao et al., 2004; Zima and Kalousová, 2005). *ADH1B*2* polymorphic allele, characterized by the presence of a restriction site for *MaeIII* in exon 3 and the substitution of arginine by histidine (CGC/CAC) in amino acid 47, yields an enzyme with 100-fold more catalytic activity than the wild type (Matsuo et al., 2006; Wu et al., 2005). On the contrary, *ALDH*2* polymorphic allele gives rise to an enzyme with no catalytic activity owing to the change of glutamic acid by lysine (GAA/AAA) at position 487 in exon 12 at nucleotide 1,510 and the subsequent loss of a *Ksp632I* restriction site (Crabb et al., 1989; Hsu et al., 1985). The substitution *C/T* at position −1,019 of *CYP2E1*, defined by the loss of an *RsaI* restriction site, originates *CYP2E1*c2 allele* featured by 10-fold greater transcriptional rate, leading to a higher amount of protein and higher enzymatic activity than the wild type (Watanabe et al., 1994). This polymorphism is also known as *CYP2E1*5B* owing to its localization at the 5′ end of the gene (Hayashi et al., 1991). Prevalence of these gene variable forms, as well as their clinical relevance associated with alcoholic liver disease has been established in diverse populations (Bataller et al., 2003; Goedde et al., 1992; Iwahashi et al., 1998). Nevertheless, conclusive results have not been obtained, probably due to clinical, genetic, and environmental variability.

As alcohol abuse is considered a major cause of cirrhosis worldwide, including Mexico (Campollo et al., 1997; Méndez-Sánchez et al., 2004), this study is aimed to analyze the association of *ADH1B*2*, *ALDH*2*, and *CYP2E1*c2* variable forms with alcoholic cirrhosis (AC) in western Mexican patients.

MATERIALS AND METHODS

Subjects

We studied 131 unrelated subjects: 90 clinically healthy control individuals (C), 34 men and 56 women, aged 42.1 ± 1.6 years, from the working staff of the hospital and 41 patients, 37 men and 4 women, aged 44.3 ± 2.2 years, previously diagnosed with AC from the Gastroenterology Service of Hospital Civil Fray Antonio Alcalde of Guadalajara (25 recurrent outpatients and 16 hospitalized patients). C group was made up with persons whose alcohol consumption was null or sporadic. Only patients with AC with a median alcohol intake of >40 g ethanol/day for more than 4 years were included. Diagnosis of liver cirrhosis was based on clinical, biochemical, and histopathological criteria. Patients with cirrhosis of virological, pharmacological, or autoimmune origin were excluded. All C and AC were of Mexican origin with a family history of at least 3 generations.

Ethical Considerations

Written informed consent was obtained from all subjects before enrollment, and the study protocol conformed to the ethical guidelines of the hospital according to the 2008 Declaration of Helsinki, adopted by the 59th World Medical Association General Assembly in Korea, 2008.

Blood Samples

About 10 ml sample of peripheral blood was drawn from each subject after a 12-hour fasting period and divided into 2 aliquots, 1 poured in a plain tube to be used for biochemical determinations and another in an EDTA-containing tube for DNA isolation.

Hepatic Function Tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin (TB), and prothrombin time (PT) were quantified according to manufacturer's protocols of commercial kits (Merck Co, Whitehouse Station, NJ). In order to minimize analytical variability, all biochemical measurements were taken at the same time both in C and AC samples.

DNA Isolation

DNA was obtained through the modified Miller's procedure (Cuevas-Covarrubias et al., 2002; Miller et al., 1988; Gordillo-Bastidas et al., 2010). Finally, DNA was resuspended in 300 μl of sterile deionized water and spectrophotometric quantification at 260/280 nm was accomplished. DNA samples were kept at −20°C for molecular analysis.

Determination of Restriction Fragment Length Polymorphism

*ADH1B*2*, *ALDH2*2,* and *CYP2E1*c2* polymorphic alleles were identified by polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism analysis. Utilized primers for *ADH1B were* sense 5′ATTTCAGGAATTTGGGTATGTT3′ and antisense 5′GGCCTAAAATCACAGGAAGG3′ (Xu et al., 1988); for *ALDH2:* sense 5′CAAATTACAGGGTCAACTGCT3′ and antisense 5′CCACACTCACAGTTTTCTCTT3′ (Takeshita et al., 1994); and for *CYP2E1:* sense 5′GTCCCTGCCACCTCACACT3′ and antisense 5′CCCTCTTCCACCTTCTATG3′ (Hayashi et al., 1991). PCR was carried out according to the protocol reported by Gordillo-Bastidas and colleagues (2010). This protocol utilizes the same conditions to amplify the 3 polymorphic fragments in different tubes in the same running of PCR, providing efficiency, reproducibility, and time saving. Amplicon sizes were 161 bp for *ADH1B*, 135 bp for *ALDH2*, and 566 bp for *CYP2E1*. Afterward, digestion of PCR products was performed following the protocols described below. Amplicons as well as restriction fragments were visualized by electrophoresis in 2% agarose stained with ethidium bromide. Amplification and digestion assays were carried out by triplicate in all cases.

ADH1B Genotyping. Digestion of PCR products was carried out in a final volume of 20 μl containing 1 μl of the amplified product, 2 U of *MaeIII* (Cat. No. 822 248; Boehringer Mannheim, Ingelheim am Rhein, Germany), 10 μl of the corresponding buffer accompanying the enzyme (2×), and deionized water. The mix was incubated for 3 hours at 55°C obtaining the following restriction pattern: 161 bp, wild genotype; 98 and 63 bp, polymorphic; 161, 98, and 63 bp, heterozygous.

ALDH2 Genotyping. One microliter of the PCR product was mixed with 1 U of *Ksp 632I* (Cat. 1081 284; Boehringer Mannheim) and 2 μl of the corresponding buffer (10×). Deionized water was added until a final volume of 20 μl was attained. The mix was incubated for 2 hours at 37°C. The restriction pattern observed was 112 and 23 bp, wild genotype; 135 bp, polymorphic; 135, 112, and 23 bp, heterozygous.

CYP2E1 Genotyping. Genotypes were determined after incubating for 3 hours at 37°C; a total volume of 20 μl of a mix made by 1 μl of PCR product, 3 U of *RsaI* (Cat. 15424-013; GibcoBRL, Carlsbad, CA), 2 μl of the corresponding buffer (10×), and deionized water. Restriction pattern observed was 143 and 423 bp, wild genotype; 566 bp, polymorphic; 566, 423, and 143 bp, heterozygous.

Statistical Analysis

Pearson chi-square test was calculated to determine frequencies differences between groups. Fisher's exact test was performed in cases where *n* value was below 10. Levene and Kolmogorov–Smirnov tests were accomplished in order to evaluate variance homogeneity and variables distribution, respectively. Student's *t* test for independent variables and Mann–Whitney *U*-test were utilized to compare quantitative variables as parametric and nonparametric tests, correspondingly. Multifactorial analysis of variance to determine variable interactions was performed. Median values along with standard error are expressed in quantitative variables. *p*-Values of 0.05 or lower were considered statistically significant.

RESULTS

Mean age was not statistically different between groups. Women were predominant in C group, meanwhile the ratio of men/women was inverted in patients with AC. However, statistically significant interaction between evaluated variables and gender was not observed. Biochemical and clinical variables describing C and AC groups are shown in Table 1. Functional liver test values exhibited statistically significant differences between C and patients with AC, assessing liver damage. According to Child-Pugh's classification, most of our patients presented severe liver cirrhosis, as 58.5% (24 of 41 patients with AC) was scored as Child C. Variables related to alcohol consumption in patients with AC are also shown in Table 1. Median age for starting alcohol drinking and alcohol consumption time was 19.1 ± 1.1 and 23.3 ± 1.9 years, respectively. Patients with AC used to drink 293.9 ± 35.3 g of alcohol daily through the ingestion of diverse alcoholic beverages, mainly tequila, brandy, or pure alcohol. Electrophoretic pattern of amplified polymorphic segments is shown in Fig. 1. Samples from Japanese controls for *ADH1B* polymorphic genotype, *ALDH2* heterozygous, and *ALDH2* polymorphic genotype were utilized, as these genotypes were not observed in the population studied. Distribution of *ADH1B*2, ALDH2*2,* and *CYP2E1*c2* polymorphic alleles and genotypes is shown in Table 2. Gender interaction was not observed when analyzing frequencies through a general linear model. Genotype distribution in C group was according to Hardy–Weinberg equilibrium ($\chi^2 = 1.24$, $p = 0.54$); except for *ALDH2*, where statistical analysis could not be performed, as individuals studied both in C and AC groups were monomorphic for the wild *ALDH2*1/*1* genotype. Only common homozygous and heterozygous *ADH1B* genotypes were observed among C and AC subjects, showing no statistically significant difference. Association between AC and *CYP2E1*c2* polymorphic allele was appreciated because frequency of *CYP2E1* heterozygous genotype and polymorphic allele was higher in patients with AC (39 and 19.5%, respectively) than in C subjects (16.7 and 10.6%, respectively), attaining statistically significant difference ($p < 0.01$ and $p < 0.05$, respectively). *CYP2E1 c2/c2* polymorphic genotype was observed in 2.2% of C group individuals, meanwhile this genotype was absent in patients with AC. Susceptibility to cirrhosis was 2.05-fold for those individuals carrying the *c2* allele, although not statistically significant (OR = 2.05; CI 95%: 0.92 to 4.50). When analyzing the association between *CYP2E1*c2* polymorphic allele and cirrhosis severity, we observed that 68.8% of the *c2* allele carrying patients with AC were Child C (11 of 16 patients with AC) and that 57.1%

Table 1. Biochemical and Clinical Variables of the Studied Mexican Clinically Healthy Individuals and Patients with Alcoholic Cirrhosis

Variable	Control (*n* = 90)	Cirrhosis (*n* = 41)	*p*
ALT (mU/ml)	5.0 ± 2.6	51.7 ± 5.9	0.001
AST (mU/ml)	8.9 ± 0.2	55.6 ± 5.8	0.001
Albumin (g/dl)	4.5 ± 0.1	2.3 ± 0.1	0.001
TB (mg/dl)	0.6 ± 0.01	8.4 ± 1.8	0.001
PT (second)	12.5 ± 0.1	18.1 ± 1.1	0.001
Child (%)			
A		3 (7.3%)	
B		14 (34.2%)	
C		24 (58.5%)	
Age of starting alcohol consumption (years)		19.1 ± 1.1	
Time of alcohol consumption (years)		23.3 ± 1.9	
Amount of alcohol ingested (g/d)		293.9 ± 35.3	

ALT, alanineamino transferase; AST, aspartate aminotransferase; TB, total bilirubin; PT, prothrombin time.

Data represent the media ± SE in all cases, except Child category, where absolute and relative frequency is specified. Differences related to gender and age were not observed in any parameter studied.

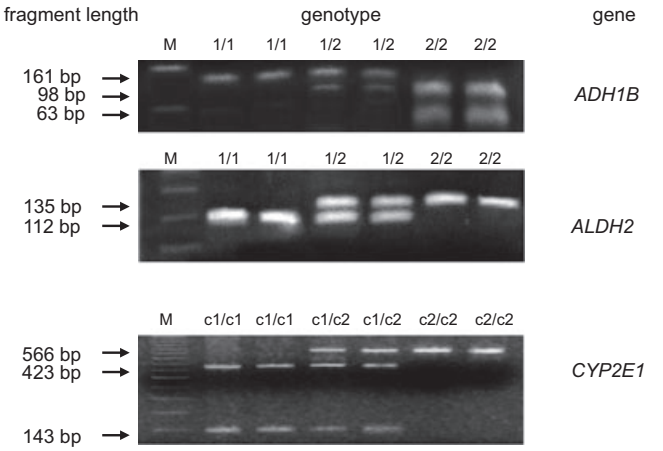

Fig. 1. Electrophoretic pattern on 2% agarose gel of amplified polymorphic segments of *ADH1B*2, ALDH*2*, and *CYP2E1*c2* alleles after digestion with *MaeIII*, *Ksp632I*, and *RsaI*, respectively. DNA fragments were stained with ethidium bromide. Samples from Japanese controls for *ADH1B* polymorphic genotype, *ALDH2* heterozygous, and *ALDH2* polymorphic genotype were utilized, as these genotypes were not observed in the population studied. M, marker of molecular weight.

Table 2. Genotype Distribution of *ADH1B*, *ALDH2*, and *CYP2E1* Polymorphisms in Western Mexican Population

Frequency	Control % (*n*)	Cirrhosis % (*n*)	*p*-Value
ADH1B genotype	(*n* = 66)	(*n* = 32)	
1/1	87.9 (58)	96.9 (31)	0.148
1/2	12.1 (8)	3.1 (1)	
2/2	0.0 (0)	0.0 (0)	
allele			
1	93.9 (124)	98.4 (63)	0.295
2	6.1 (8)	1.6 (1)	
ALDH2 genotype	(*n* = 79)	(*n* = 41)	
1/1	100.0 (79)	100.0 (41)	NC
1/2	0.0 (0)	0.0 (0)	
2/2	0.0 (0)	0.0 (0)	
allele			
1	100.0 (158)	100.0 (82)	NC
2	0.0 (0)	0.0 (0)	
CYP2E1 genotype	(*n* = 90)	(*n* = 41)	
c1/c1	81.1 (73)	61.0 (25)	0.011*
c1/c2	16.7 (15)	39.0 (16)	
c2/c2	2.2 (2)	0.0 (0)	
allele			
c1	89.4 (161)	80.5 (66)	0.048*
c2	10.6 (19)	19.5 (16)	

*$p < 0.05$ statistically significant; NC, not calculated due to the existence of data equal to zero.

Distribution was not associated to gender or age.

of the hospitalized patients who died during the study (4 of 7 AC Child C patients), presented the *c2* allele as *c1/c2* heterozygous genotype (data not shown), yet not reaching statistical significance. Besides, liver function evaluated through ALT, AST, TB, and PT determination was worse in *c1/c2* patients with AC than in the *c1/c1* patients, reaching statistically significant differences in TB and PT values, as shown in Fig. 2 ($p < 0.05$).

DISCUSSION

Alcohol abuse constitutes a worldwide major etiological factor for liver cirrhosis (Campollo et al., 1997; Douds et al., 2003; Méndez-Sánchez et al., 2004; Takada and Tsutsumi, 1995). In an effort to better understand the bases underlying this nosological entity, a variety of studies describing possible molecular and pathophysiological factors involved have been accomplished (Bastidas-Ramírez et al., 2002; Gordillo-Bastidas et al., 2010; Hernández-Nazará et al., 2008; Nuño-González et al., 2005). Ethnicity influence over differential susceptibility to alcoholic liver disease has widely been determined (Douds et al., 2003). Besides, selection of a candidate gene for an association case–control study is usually based on biological plausibility in chosen genes that play a putative role in the pathogenesis of the studied disease (Day, 2003). Only genetic variants that result in altered transcription, RNA stability, or protein function are likely to modulate disease progression (Daly, 2003; Stickel and Österreicher, 2006). In vivo and in vitro studies have emerged regarding the functional impact of *ADH1B*2*, *ALDH2*2*, and *CYP2E1*c2* variant alleles. *ADH1B*2* and *ALDH2*2* constitute variations affecting the coding region of the gene, leading to a protein with higher or null enzymatic activity compared with the protein coded by the wild type allele, *ADH1B*1* **(**Lee et al., 2006; Zakhari and Li, 2007**)** or *ALDH2*1* **(**Chen et al., 2009; Wall et al., 1997; Zakhari and Li, 2007**),** respectively, resulting in acetaldehyde accumulation. Asians are the prevalent

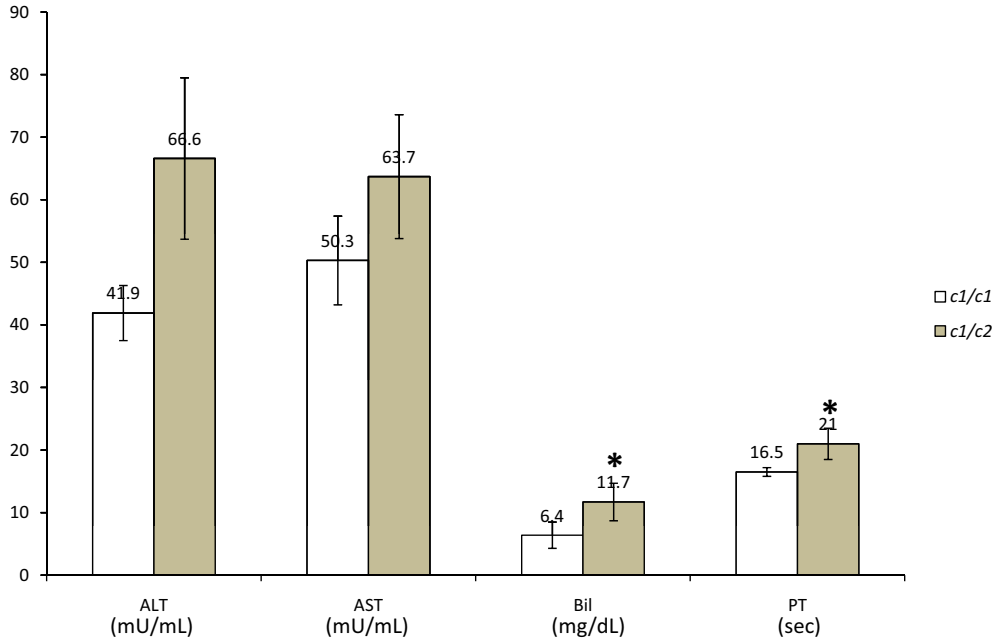

Fig. 2. *CYP2E1* genotype and functional liver tests in patients with alcoholic cirrhosis. Polymorphic *c2/c2* genotype was not observed in the patients studied. Statistical differences regarding to gender or age were not observed. Data represent the media ± SE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; PT, prothrombin time. *$p < 0.05$.

population for these 2 polymorphisms (Matsuo et al., 2006; Yang et al., 2007), who also exhibit association with susceptibility to AC and alcoholism (Chao et al., 1994; Yamauchi et al., 1995a,b). It is important to state that Mexican population included in our study was from West Mexico. Contrary to data reported in Asians, a very low frequency of the *ADH1B*2* variant allele and no association with AC susceptibility was appreciated. On the same way, monomorphism, thus lack of association between *ALDH2*2* allele and AC susceptibility was observed, because all subjects analyzed were genotyped as *ALDH2*1/1*. According to our results, it seems that *ADH1B*2* and *ALDH2*2* variant alleles do not participate in the pathogenesis of AC in the population studied, as their frequency is very low or totally absent, correspondingly. Similar results have been obtained by other authors in diverse populations, like in French patients with cirrhosis (Couzigou et al., 1990), Spanish (Vidal et al., 2004), and Korean men (Lee et al., 2001).

*CYP2E1*c2* polymorphic variant—although not affecting the protein primary structure, as it results from a nucleotide variation located at the promoter region of the gene—also constitutes a relevant polymorphism for its influence in increasing the transcriptional rate, protein amount and enzyme activity (Hayashi et al., 1991; Watanabe et al., 1994), leading to *CYP2E1* overexpression, free radical production, and pathology association (Cichoz-Lach et al., 2006; Gao et al., 2007; Lieber, 2004b; Ueno et al., 1996). Functional significance of this polymorphism may be attributed to its localization in presumed DNA responding element for hepatic transcription factor 1 (Hayashi et al., 1991; Watanabe et al., 1994). Major frequencies of *CYP2E1*c2* polymorphic allele corresponded to Chinese (30%; Tan et al., 2000) and Japanese populations (30.9%; Morita et al., 2009) until recently, when the highest frequency reported is in Huichols (51%; Gordillo-Bastidas et al., 2010), an indigenous group from Western Central Mexico. Various studies have demonstrated that carrying *c2* allele may lead to increased risk to develop alcoholic liver disease (Cichoz-Lach et al., 2006; Konishi et al., 2003; Pirmohamed et al., 1995). Moreover, Monzoni and colleagues (2001) have documented that in Italian population, *c2* allele constitutes an independent risk factor in alcohol abusers, representing a risk 3.2 times higher (CI 1.5 to 6.7) of developing AC. Nevertheless, association has not been observed by other authors (Agundez et al., 1996; Wong et al., 2000). In this study, significant association of *CYP2E1*c2* allele and *c1/c2* genotype with AC susceptibility, together with severity of liver disease by biochemically assessing liver function, was observed. A trend to higher values of ALT and AST was shown in patients with the *c2* allele; meanwhile, TB and PT presented significant higher values in heterozygous patients when compared with common genotype-carrying patients. TB, as well as PT are biomarkers accounting for Child-Pugh score that constitutes a useful tool in the evaluation of cirrhosis progression considering the synthetic and elimination functions of the liver (Durand and Valla, 2005). Child-Pugh score is categorized according to the increasing stage of decompensation of cirrhosis from Child A to Child C. In this context, *CYP2E1*c2* allele is probably playing an important role in the prognosis of liver disease as well, because most of the *CYP2E1*c2* allele carrying patients with AC scored as Child C and the majority of patients who died presented the polymorphic allele, although statistical significance was not reached. With this regard, the participation of *c2* allele as an independent biomarker of risk and poor outcome in alcohol-related head and neck squamous cell carcinoma patients has recently been described (Ribeiro-Olivieri et al., 2009). Likewise, proposals about the possible utility of P4502E1 expression to predict alcohol-induced toxicity in patients with AC (Khan et al., 2011; Webb et al., 2011), where polymorphisms may be playing an important role, are currently the interest of several researching groups.

Liver damage as a result of alcohol consumption has been attributed mainly to 2 distinct factors: acetaldehyde accumulation and oxidative stress. *CYP2E1*c2* allele could be particularly important in patients with AC, as CYP2E1 has been suggested to be a major contributor to ethanol-induced oxidant stress and ethanol-induced liver injury through acetaldehyde accumulation (Lu and Cederbaum, 2008; Nanji et al., 1994). Besides, CYP2E1 in patients with AC is probably not only induced by alcohol consumption, but also by a variety of other metabolic or nutritional conditions, like obesity and a low carbohydrate-rich lipid diet (Lindros and Jarvelainen, 1998; Raucy et al., 1991), or fasting and prolonged starvation (Hong et al., 1987; Johansson et al., 1990), as it has been reported, promoting liver damage. All these conditions are likely to occur in patients suffering from AC (Marceau et al., 1999; Marrero et al., 2005; Mezey, 1998; Poonawala et al., 2000), probably worsening liver disease when associated to *CYP2E1*c2* polymorphic allele. However, these associations deserve further evaluation, as we did not register body mass index or nutritional variables. On the other hand, the possible predictive value of *CYP2E1*c2* allele as a marker of prognosis and severity of AC in individuals particularly sensitive to ethanol-induced oxidant stress and liver injury suggested in this study, also need deeper investigation.

A meta-analysis performed by Zintzaras and colleagues (2006), finding no association between *CYP2E1*c2* allele and alcoholic liver disease, has been published. However, only 1 alcoholism association study performed in Mexicans living in Los Angeles was considered. With this regard, association studies in Mexican population dealing with genes coding for alcohol-metabolizing enzymes and cirrhosis are scarce. Moreover, results of gene association studies performed in Mexican population may vary from region to region of the country owing to the great heterogeneity documented (Aceves et al., 2006; Hernández-Nazará et al., 2008), turning our report an important contribution in the pathophysiological field of this worldwide public health problem.

The significant higher frequency of *CYP2E1*c2* allele and *c1/c2* genotype observed in patients with AC, together with a worse outcome evaluated through TB and PT when compared with healthy controls, may indicate the participation of *c2*

allele in the development of AC in the studied population. Nevertheless, some apparent weaknesses of the study are commented below. Notwithstanding usually most chronic drinkers have revealed some degree of liver damage (Teli et al., 1995), comparison of our results not only with healthy subjects but also with a control group conformed by alcoholic patients apparently free of liver damage would be suitable. On the other hand, regarding gender disproportion between C and AC groups, gender interaction with all the parameters analyzed in this study was discarded through multifactorial analysis of variance. However, in order to guarantee variable interaction control, increasing the size of the sample avoiding female/male uneven distribution between groups would better support our results.

Alcoholic liver disease is undoubtedly a multifactorial process (Lu and Cederbaum, 2008), where discrepancy in ethnicity, liver damage extent, life style, nutritional, and metabolic factors may explain controversial results. Probably, the virtual absence of the so-called alcoholism protecting alleles (Bosron and Li, 1986), *ADH1B*2* and *ALDH2*2*, together with the AC susceptibility associated allele *CYP2E1*c2* observed in our study, may be contributing to the public health problem of AC in Mexican population. Nevertheless, further studies should be accomplished in order to elucidate the complex orchestration of the multiple factors involved in the pathophysiological process of AC.

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