Genetic polymorphisms of \textit{ADH1B}, \textit{ADH1C} and \textit{ALDH2} in Turkish alcoholics: lack of association with alcoholism and alcoholic cirrhosis

Sezgin Vatansever$^1$, Fatih Tekin$^{*1}$, Esin Salman$^1$, Ender Altintoprak$^2$, Hakan Coskunol$^3$, Ulus Salih Akarca$^2$

$^1$Department of Gastroenterology and $^2$Department of Psychiatry, Ege University Medical School, Izmir, Turkey

ABSTRACT

No data exists regarding the alcohol dehydrogenase (\textit{ADH}) and aldehyde dehydrogenase (\textit{ALDH}) gene polymorphisms in Turkish alcoholic cirrhotics. We studied the polymorphisms of \textit{ADH1B}, \textit{ADH1C} and \textit{ALDH2} genes in alcoholic cirrhotics and compared the results with non-cirrhotic alcoholics and healthy volunteers. Overall, 237 subjects were included for the study: 156 alcoholic patients (78 cirrhotics, 78 non-cirrhotic alcoholics) and 81 healthy volunteers. Three different single-nucleotide-polymorphism genotyping methods were used. \textit{ADH1C} genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism method. The identified \textit{ADH1C} genotypes were named according to the presence or absence of the enzyme restriction sites. \textit{ADH1B} (Arg47Hys) genotyping was performed using the allele specific primer extension method, and \textit{ALDH2} (Glu497Lys) genotyping was performed by a multiplex polymerase chain reaction using two allele-specific primer pairs. For \textit{ADH1B}, the frequency of allele ‘1’ in the cirrhotics, non-cirrhotic alcoholics and healthy volunteers was 97.4%, 94.9% and 99.4%, respectively. For \textit{ADH1C}, the frequency of allele ‘1’ in the cirrhotics, non-cirrhotic alcoholics and healthy volunteers was 47%, 36.3% and 45%, respectively. There was no statistical difference between the groups for \textit{ADH1C} (p>0.05). All alcoholic and non-alcoholic subjects (100%) had the allele ‘1’ for \textit{ALDH2}. The obtained results for \textit{ADH1B}, \textit{ADH1C}, and \textit{ALDH2} gene polymorphisms in the present study are similar to the results of Caucasian studies. \textit{ADH1B} and \textit{ADH1C} genetic variations are not related to the development of alcoholism or susceptibility to alcoholic cirrhosis. \textit{ALDH2} gene has no genetic variation in the Turkish population.

KEYWORDS: alcohol gene polymorphism; alcoholism; cirrhosis; Turkey

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INTRODUCTION

Ethanol is mostly metabolized in the liver by the contributions of alcohol dehydrogenase (\textit{ADH}) and aldehyde dehydrogenase (\textit{ALDH}). Alcohol is oxidized to acetaldehyde by the \textit{ADH} enzymes, and acetaldehyde is further oxidized to acetate by the \textit{ALDH} enzymes. Acetaldehyde, a highly toxic metabolite of ethanol, has a very important effect on the pathogenesis of alcoholic liver disease and alcohol dependence. Studies proved that accumulation of acetaldehyde in blood induces unpleasant effects such as flushing, headache, tachycardia, and hypotension [1,2]. Because these factors limit continual drinking, they may considerably prevent alcohol dependence. Further, these data suggested that genetically determined variations in an individual’s ability to metabolize alcohol might influence the prevalence of alcoholic liver diseases. Both the \textit{ADH} and \textit{ALDH} enzymes exhibit genetic polymorphisms [3-5]. The rates of metabolic pathways responsible for converting alcohol to acetaldehyde and acetaldehyde to acetate are mostly influenced by functional polymorphisms on \textit{ADH1B} (previously called \textit{ADH2}), \textit{ADH1C} (previously called \textit{ADH3}), and \textit{ALDH2} [5-7].

In addition to \textit{ADH} and \textit{ALDH} enzymes, cytochrome \textit{P450 2E1} (\textit{CYP2E1}) is also a pathway of ethanol oxidation. \textit{CYP2E1} catalyzes two-electron oxidation of ethanol to acetaldehyde, and also ethanol to acetate [8]. \textit{CYP2E1} has a low ethanol catalytic efficiency when compared to \textit{ADH}, and is responsible for only a small part of the total ethanol metabolism. However, \textit{CYP2E1} is an inducible enzyme by ethanol, and the most significant role of \textit{CYP2E1} is its adaptive response to high blood ethanol levels with a corresponding acceleration of ethanol metabolism [9]. The gene of this enzyme is polymorphic, and the genetic variation is associated with alcohol dependence [10-12].
Alcoholism is an important cause of chronic liver diseases, but only 10% to 20% of alcoholics develop cirrhosis [13]. While a group of drinkers do not develop cirrhosis, another group who possibly consume less alcohol can have considerable liver damage. In Asians, the $ALDH2^*$2 allele, which encodes for an inactive $ALDH2$ form, appears to protect against alcoholism [14,15]. Furthermore, alcoholics with this inactive allele may be at a greater risk of advanced alcoholic liver disease [16,17]. However, the $ALDH2^*$2 allele has not been found in Caucasians [15,18]. In Asians, polymorphisms of the $ADH1B$ and $ADH1C$ genes were associated with the development of alcoholism and susceptibility to alcoholic liver cirrhosis [16,17,19,20]. However, the relationship between polymorphisms at the $ADH1B$ and $ADH1C$ loci, and the individual predisposition to alcoholic liver disease in Caucasians is controversial [21,22]. On the other hand, no data exists regarding the possible relationship between the polymorphism of genes encoding alcohol metabolizing enzymes and Turkish alcoholic cirrhotics. Thus, we studied the polymorphisms of $ADH1B$, $ADH1C$ and $ALDH2$ genes in Turkish alcoholic cirrhotics, and compared the results with non-cirrhotic alcoholics and healthy volunteers.

MATERIALS AND METHODS

Subjects

We studied 81 non-alcoholic healthy volunteers as a control group (mean age±S.D.: 36±17.5 years, 27 male, 54 female), and 156 consecutive alcoholic patients of Turkish origin (mean age±S.D.: 46±14.6 years, 145 male, 11 female) who were admitted to the department of Gastroenterology or to the Alcohol Dependence Unit in department of Psychiatry at the hospital of Ege University Medical School, Izmir, Turkey. Alcoholic patients were classified into two groups according to their final diagnosis: cirrhotics and non-cirrhotics. Subjects whose alcohol consumption was ≥40 gr/day for longer than 10 years were considered alcoholics. The criteria for diagnosing alcoholic cirrhosis were the presence of alcohol consumption ≥40 gr/day for longer than 10 years, signs of advanced liver disease including jaundice, hepatic encephalopathy, ascites, portal hypertensive bleeding, or splenomegaly; the presence of laboratory abnormalities such as low serum albumin, and/or prolonged prothrombin time, and the presence of radiologic features of cirrhosis such as a nodular liver surface, ascites, and splenomegaly. Healthy volunteers were hospital workers and students of Ege University Medical School who had no history of alcoholism or chronic liver disease. The present study was performed in accordance with the ethical standards and the Declaration of Helsinki. Written informed consent was obtained from each subject.

DNA extraction and genotyping

DNA was extracted from whole anticoagulated blood using a commercial kit (Invisorb Spin Blood DNA Purification Kit, STRATEC Biomedical AG, Birkenfeld, Germany) and genotypes were determined using different PCR based methods. Literature was searched for genotyping methods. Several methods were performed in our laboratory and the best methods, which could be optimized in our laboratory, were selected. Bioron GmbH, Ludwigsafen, Germany, provided Taq polymerase and restriction enzyme primers were provided by MWG Biotech AG, Ebersberg, Germany.

$ADH1B$ genotyping

The $ADH1B$ single-nucleotide polymorphism studied is a G to A transition in exon 3, which changes arginine to histidine at residue 47 (Arg47Hys). $ADH1B$ genotyping was carried out using an allele specific primer extension protocol according to a published method [23]. The PCR reaction reagents mix contained 30-100 ng Genomic DNA, 0.18 mM dNTPs, 10 pmol of each primer, 0.5 Units of Hot Start Taq DNA polymerase, and 2.5 μl of 10X PCR Buffer including 25 Mm MgCl$_2$. The cycling conditions were as follows: initial denaturation 94°C for 5 min; 30 cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds; and final elongation at 72°C for 5 min. Arginine specific primers: forward: 5'-TCT GTA GAT GGT GGC TGT AGG AAT CTG CCA-3', the reverse primer was the same as arginine's. Primers were provided by MWG Biotech AG, Ebersberg, Germany. A positive band at 538 bp with either arginine or histidine primers indicated genotype 2.1 or 2.2, respectively.

$ADH1C$ genotyping

The $ADH1C$ genotype was determined according to the method of Vidal et al. [24], with minor modifications. The amplification reaction was carried out in a final volume of 25 μl containing 25 mM MgCl$_2$, 0.18 mM dNTPs, 10 pmol of each primer and 0.5 Units of Hot Start Taq DNA polymerase. 100 ng genomic DNA was amplified for 35 cycles. The cycling conditions were as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, 57°C for 45 sec, and 63°C for 1 min; and final elongation at 63°C for 5 min. The primers used were 5'-GCTTTAAGAGTAAATAATCTGTCCCC-3' and 5'-AATCTACCTCTTTCGCCAGGCC-3' for $ADH1C$ genotypes. For allele detection, aliquots of amplified DNA product were digested with SspI at 37°C for 16 hours. Digestion products were run on 2% 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. G/G=*1/*1, G/A=*1/*2, A/A=*2/*2 are homozygotes for the absence of site (146 bp), heterozygotes (63-83 and 146 bp), and homozygotes for the presence of site (63-83 bp) respectively.

**ADH1B genotyping**

*ADH1B* gene located in 12q24.2 has a G to A polymorphism (Glu487Lys). *ADH1B* genotyping was performed with multiplex PCR, using the method of Tamakoshi et al. [25]. Two forward and two reverse primers (F1:5’-TGC TAT GAT GTG TTT GGA GCC-3’, F2: 5’-GGGCTG CAGGCATACTA-3’, R1: 5’- CCA CAC ACC TAC ACG TTT CAC TTT C-3’, R2: 5’-GGCTCCAGGACCA-3’) were used. The two outer primers (F1 and R2) amplified a 176 bp fragment in all samples. For the 487 Glu (*ADH1B*’*) a 19 bp fragment was amplified using primers F1 and R1; for the 487 Lys (*ADH1B*’2) a 98 bp fragment was amplified using primers F2 and R2. PCRs were performed in a 25 μl reaction mixture containing 10× PCR buffer, 0.18 mM dNTPs, 25 Mm MgCl2, 10 pmol each primer of forward and reverse primers, 0.5 Units of Hot Start Taq DNA polymerase, and 100 ng Genomic DNA. The cycling conditions were as follows: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 30 seconds, 63°C for 1 min, and 72°C for 1 min; and final elongation at 72°C for 5 min.

**Statistical Analysis**

Chi-square tests were performed to compare the genotype distributions of *ADH1B* (*1/*1, *1/*2, *2/*2) and *ADH1C* (*1/*1, *1/*2, *2/*2) genes and *1 and *2 allele frequencies between healthy volunteers versus alcoholics and non-cirrhotic alcoholics versus alcoholic cirrhosis. A *p* value less than 0.05 was accepted as statistically significant. All statistical analyses were performed using SPSS 11.0.

**RESULTS**

Among 156 alcoholics, 78 (50%) patients were diagnosed as cirrhotic. *ADH1B* genotypes were studied in 156 alcoholics and 81 healthy volunteers. The *ADH1B*’1/*1 genotype was the most common type of all *ADH1B* genotypes in both alcoholic and non-alcoholic groups. There was no statistical difference between alcoholic cirrhosis, non-cirrhotic alcoholics and non-alcoholic groups for *ADH1B* genotyping (Table 1a,b).

*ADH1C* genotypes were studied in 141 alcoholics and 80 healthy volunteers. DNA extraction could not be performed in 15 alcoholics and in 1 healthy volunteer due to technical problems. Healthy volunteers had statistically higher frequencies of *ADH1C*’1/*2 (*p*=0.0003) and *ADH1C*’2/*2 (*p*=0.0112) than the

**DISCUSSION**

We found that the allele frequencies of *ADH1B* and *ADH1C* showed no statistically significant difference among Turkish patients with alcoholic cirrhosis, non-cirrhotic alcoholics, and healthy nondrinkers. These results indicate that there exists no association between the *ADH1B* and *ADH1C* gene polymorphisms and susceptibility to alcohol dependency or development of alcoholic cirrhosis. To our knowledge, this is the first study investigating *ADH1B*, *ADH1C*, and *ALDH2* gene polymorphisms in Turkish alcoholic cirrhosis. Our results were consistent with a previously published meta-analysis, which reviewed studies on associations between polymorphisms in genes coding for *ADH6* and *ALDHs* and retrieved 50 case control studies between 1990 and 2004 [21]. In this meta-analysis, no overall association of any of the tested polymorphisms with alcoholic cirrhosis was detected.

Only two studies from Turkey have been previously performed to investigate the polymorphisms in the genes

| TABLE 1A. Genotype distribution and allele frequency of *ADH1B* gene in Turkish alcoholics and controls |
|-----------------|-----------------|-----------------|
| Groups          | Genotype distribution, n (%) | Allele frequency (%) |
|                 | *ADH1B*’1/*1 | *ADH1B*’1/*2 | *ADH1B*’2/*2 |
| Alcoholics       |                |                |              |
| (n=156)         | 145 (91.0)    | 10 (6.4)       | 1 (0.6)      |
| Controls         |                |                |              |
| (n=81)          | 50 (97.4)     | 145 (93.0)     | 94 (93.0)    |
| p value          | ns             | ns             | ns           |
| ns: not significant |

| TABLE 1B. Genotype distribution and allele frequency of *ADH1B* gene in Turkish alcoholic cirrhotics and alcoholic non-cirrhotics |
|-----------------|-----------------|-----------------|
| Groups          | Genotype distribution, n (%) | Allele frequency (%) |
|                 | *ADH1B*’1/*1 | *ADH1B*’1/*2 | *ADH1B*’2/*2 |
| Cirrhotics       |                |                |              |
| (n=78)          | 74 (94.9)     | 4 (5.1)        | 0 (0)        |
| Non-cirrhotics  |                |                |              |
| (n=78)          | 71 (91.0)     | 6 (7.7)        | 1 (1.3)      |
| p value          | ns             | ns             | ns           |
| ns: not significant |
TABLE 2A. Genotype distribution and allele frequency of ADH1C gene in Turkish alcoholics and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype distribution, n (%)</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADH1C*1/*1</td>
<td>ADH1C*1/*2</td>
</tr>
<tr>
<td>Alcoholics (n=141)</td>
<td>21 (14.9)</td>
<td>75 (53.2)</td>
</tr>
<tr>
<td>Controls (n=80)</td>
<td>5 (6.2)</td>
<td>62 (77.5)</td>
</tr>
<tr>
<td>p value</td>
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<td>0.0003</td>
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</table>

ns: not significant

TABLE 2B. Genotype distribution and allele frequency of ADH1C gene in Turkish alcoholic cirrhotics and alcoholic non-cirrhotics

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype distribution, n (%)</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADH1C*1/*1</td>
<td>ADH1C*1/*2</td>
</tr>
<tr>
<td>Cirrhotics (n=68)</td>
<td>14 (20.6)</td>
<td>36 (52.9)</td>
</tr>
<tr>
<td>Non-cirrhotics (n=73)</td>
<td>7 (9.6)</td>
<td>39 (53.4)</td>
</tr>
<tr>
<td>p value</td>
<td>ns</td>
<td>ns</td>
</tr>
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</table>

ns: not significant

encoding alcohol metabolizing enzymes. Among these studies, Kayaalti and Soylmezoglu [26] found that the allele frequencies of ADH1B*1 and ALDH2*1 in healthy volunteers were 87.5% and 100%, respectively. ADH1C gene polymorphisms were not studied, and only healthy volunteers were included in that study. The other study by Aktas et al. [27] was performed in healthy volunteers and alcoholics without any subgroup analysis of cirrhotics. They found that the allele frequencies of ADH1B*1 and ADH1C*1 in healthy volunteers versus alcoholics were 88.1% vs. 95.9% and 31.5% vs. 26%, respectively. ALDH2 gene polymorphisms were not included in this study. Our results showed that the distribution of the allele ‘*1 and allele ‘*2 variants in the analyzed healthy volunteers was: ADH1B 99.4% and 0.6%; ADH1C 45% and 55%; and ALDH2 100% and 0% respectively. Our results were consistent with the other two previously published studies from Turkey. In addition, we compared the allelic frequencies observed in the different groups defined (controls vs. alcoholics, non-cirrhotic alcoholics vs. cirrhotics), and in no cases were the differences found to be significant. The data of three studies from Turkey are summarized in Table 4. If all the results of the studies from Turkey are taken into consideration, it is inferred that Turkish people have the inherited variations of ADH and ALDH genes, which do not protect them from alcohol sensitivity and dependency. This case is consistent with the white race in the world including European people but not consistent with Asian people.

The ADH1B*1 allele was more common in both alcoholic and non-alcoholic Turkish people. This is consistent with the literature demonstrating that ADH1B*1 is the most common type of ADH1B among world population including both white and black races. The ADH1B*1 allele converts ethanol to acetaldehyde at a slow rate, hence, it seems that Turkish people have a tendency for alcohol tolerance. Our results showed that the ADH1C*2 allele was not statistically different between alcoholic cirrhotics and non-cirrhotics. In this study, the ADH1C*2 allele (inactive allele) was not observed in either control or alcoholic subjects. So, it can be concluded that the ADH1C*2 inactive allele is not found in Turkish people like most of Caucasian race. The presence of the ADH1C*1 allele or the absence of the ADH1C*2 allele supports the data that the Turkish population is less susceptible to alcohol dependency.

CONCLUSION

In conclusion, allele ‘*1 and ‘*2 frequencies of ADH1B, ADH1C and ALDH2 genes in the Turkish population are similar to those found in Caucasian populations. Our data show that ADH1B and ADH1C genotypes are not related to the individual risk of alcoholism or the development of alcoholic

TABLE 4. Allele frequencies of ADH1B, ADH1C, and ALDH2 of 3 studies from Turkey

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population, n</th>
<th>Studied genes</th>
<th>Allele frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Healthy volunteers (n=211)</td>
<td>ADH1B</td>
<td>‘*1=87.5%, ‘*2=12.5%</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (n=88 for ADH1B, n=100 for ADH1C)</td>
<td>ALDH2</td>
<td>‘*1=100%, ‘*2=0%</td>
</tr>
<tr>
<td>27</td>
<td>Alcoholics (n=81 for ADH1B and ALDH, n=80 for ADH1C)</td>
<td>ADH1B</td>
<td>‘*1=88.1%, ‘*2=11.9%</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (n=73 for ADH1B, n=75 for ADH1C)</td>
<td>ADH1C</td>
<td>‘*1=31.5%, ‘*2=68.5%</td>
</tr>
<tr>
<td></td>
<td>Alcoholics (n=78 for ADH1B, n=73 for ADH1C)</td>
<td>ADH1B</td>
<td>‘*1=95.9%, ‘*2=4.1%</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (n=78 for ADH1B, n=73 for ADH1C)</td>
<td>ALDH2</td>
<td>‘*1=26%, ‘*2=74%</td>
</tr>
<tr>
<td></td>
<td>Alcoholics (n=78 for ADH1B, n=68 for ADH1C)</td>
<td>ADH1B</td>
<td>‘*1=98.6%, ‘*2=1.4%</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (n=78 for ADH1B, n=73 for ADH1C)</td>
<td>ADH1C</td>
<td>‘*1=94.9%, ‘*2=5.1%</td>
</tr>
<tr>
<td></td>
<td>Alcoholics (n=78 for ADH1B, n=68 for ADH1C)</td>
<td>ALDH2</td>
<td>‘*1=100%, ‘*2=0%</td>
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<tr>
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<td>Alcoholics (n=78 for ADH1B, n=68 for ADH1C)</td>
<td>ADH1B</td>
<td>‘*1=97.4%, ‘*2=2.6%</td>
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<tr>
<td></td>
<td>Healthy volunteers (n=78 for ADH1B, n=73 for ADH1C)</td>
<td>ADH1C</td>
<td>‘*1=47%, ‘*2=53%</td>
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<td>Alcoholics (n=78 for ADH1B, n=68 for ADH1C)</td>
<td>ALDH2</td>
<td>‘*1=100%, ‘*2=0%</td>
</tr>
</tbody>
</table>
cirrhosis in the Turkish population. We suggest that further studies of the genetic factors involved in the risk of alcoholism and development of cirrhosis may be needed.

DECLARATION OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES