Genetic polymorphisms of *ADH1B*, *ADH1C* and *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[2], Ulus Salih Akarca[1]

[1]Department of Gastroenterology and [2]Department of Psychiatry, Ege University Medical School, Izmir, Turkey

ABSTRACT

No data exists regarding the alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) gene polymorphisms in Turkish alcoholic cirrhotics. We studied the polymorphisms of *ADH1B, ADH1C* and *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. *ADH1C* genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism method. The identified *ADH1C* genotypes were named according to the presence or absence of the enzyme restriction sites. *ADH1B* (Arg47Hys) genotyping was performed using the allele specific primer extension method, and *ALDH2* (Glu487Lys) genotyping was performed by a multiplex polymerase chain reaction using two allele-specific primer pairs. For *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 *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 *ADH1B* and *ADH1C* ($p>0.05$). All alcoholic and non-alcoholic subjects (100%) had the allele *1 for *ALDH2*. The obtained results for *ADH1B, ADH1C*, and *ALDH* gene polymorphisms in the present study are similar to the results of Caucasian studies. *ADH1B* and *ADH1C* genetic variations are not related to the development of alcoholism or susceptibility to alcoholic cirrhosis. *ALDH2* gene has no genetic variation in the Turkish population.

KEYWORDS: alcohol; gene polymorphism; alcoholism; cirrhosis; Turkey
DOI: http://dx.doi.org/10.17305/bjbms.2015.242
Bosn J Basic Med Sci. 2015;15(2):37-41. © 2015 ABMSFBIH

INTRODUCTION

Ethanol is mostly metabolized in the liver by the contributions of alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*). Alcohol is oxidized to acetaldehyde by the *ADH* enzymes, and acetaldehyde is further oxidized to acetate by the *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 *ADH* and *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 *ADH1B* (previously called *ADH2*), *ADH1C* (previously called *ADH3*), and *ALDH2* [5-7].

In addition to *ADH* and *ALDH* enzymes, cytochrome *P450 2E1* (*CYP2E1*) is also a pathway of ethanol oxidation. *CYP2E1* catalyzes two-electron oxidation of ethanol to acetaldehyde, and also ethanol to acetate [8]. *CYP2E1* has a low ethanol catalytic efficiency when compared to *ADH*, and is responsible for only a small part of the total ethanol metabolism. However, *CYP2E1* is an inducible enzyme by ethanol, and the most significant role of *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].

*Corresponding Author: Dr. Fatih Tekin,
Gastroenteroloji Bilim Dali, Ege Universitesi Tip Fakultesi, Bornova 35100, Izmir, TURKEY. Phone: +90-232-3904397.
E-mail: drtekinfatih@gmail.com

Submitted: 24 November 2014 / Accepted: 18 December 2014

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.: 36.3±17.5 years, 27 male, 54 female), and 156 consecutive alcoholic patients of Turkish origin (mean age±S.D.: 46.6±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, Ludwigshafen, 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 ACG-3,' the reverse: 5'-TAC TTT TTT TCC CTC CTC CCG TTT CTA CTT CTA-3'; histidine 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 25µl 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'-AATCTACCTCTTTCCAGAGC-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.

ALDH genotyping

ALDH2 gene located in 12q24.2 has a G to A polymorphism (Glu487Lys). *ALDH* 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 CAGGCATACACTA-3', R1: 5' - C C C A C A C T C A C A G T T T T C A C T T C - 3', R2: 5'-GGCTCCGAGCCACCA-3') were used. The two outer primers (F1 and R2) amplified a 176 bp fragment in all samples. For the 487 Glu (ALDH2*1) a 119 bp fragment was amplified using primers F1 and R1; for the 487 Lys (ALDH2*2) a 98 bp fragment was amplified using primers F2 and R2. PCRs were performed in a 25µl reaction mixture containing 10× PCR buffer, 0.18 mM dNTPs, 25Mm 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 cirrhotics. 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 cirrhotics, 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 alcoholics. However, the overall allele frequency of *ADH1C*1* and *ADH1C*2* was not statistically different between these two groups. Furthermore, genotype distribution and allele frequency of *ADH1C* was not statistically different between alcoholic cirrhotics and non-cirrhotic alcoholics (Table 2a,b).

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*	*1	*2
Alcoholics (n=156)	145 (93.0)	10 (6.4)	1 (0.6)	96.2	3.8
Controls (n=81)	80 (98.7)	1 (1.3)	0 (0)	99.4	0.6
p value	ns	ns	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*	*1	*2
Cirrhotics (n=78)	74 (94.9)	4 (5.1)	0 (0)	97.4	2.6
Non-cirrhotics (n=78)	71 (91.0)	6 (7.7)	1 (1.3)	94.9	5.1
p value	ns	ns	ns	ns	ns

ns: not significant

ALDH2 genotypes were studied in 156 alcoholics and 80 healthy volunteers. DNA extraction could not be performed in one healthy volunteer due to technical problems. In the all alcoholic and non-alcoholic subjects examined, the frequency of *ALDH2*1/*1* was found 100%. We have not detected any polymorphism at the *ALDH2* locus (Table 3).

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 cirrhotics. Our results were consistent with a previously published meta-analysis, which reviewed studies on associations between polymorphisms in genes coding for *ADHs* 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 2A. Genotype distribution and allele frequency of *ADH1C* gene in Turkish alcoholics and controls

Groups	Genotype distribution, n (%)			Allele frequency (%)	
	*ADH1C*1/*1*	*ADH1C*1/*2*	*ADH1C*2/*2*	*1	*2
Alcoholics (n=141)	21 (14.9)	75 (53.2)	45 (31.9)	41.5	58.5
Controls (n=80)	5 (6.2)	62 (77.5)	13 (16.3)	45.0	55.0
p value	ns	0.0003	0.0112	ns	ns

ns: not significant

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

Groups	Genotype distribution, n (%)			Allele frequency (%)	
	*ADH1C*1/*1*	*ADH1C*1/*2*	*ADH1C*2/*2*	*1	*2
Cirrhotics (n=68)	14 (20.6)	36 (52.9)	18 (26.5)	47.0	53.0
Non-cirrhotics (n=73)	7 (9.6)	39 (53.4)	27 (37.0)	36.3	63.7
p value	ns	ns	ns	ns	ns

ns: not significant

TABLE 3. Genotype distribution and allele frequency of *ALDH2* gene in Turkish alcoholics and controls

Groups	Genotype distribution, n (%)			Allele frequency (%)	
	*ALDH2*1/*1*	*ALDH2*1/*2*	*ALDH2*2/*2*	*1	*2
Alcoholics (n=141)	141 (100)	0 (0)	0 (0)	100	0
Controls (n=81)	81 (100)	0 (0)	0 (0)	100	0

encoding alcohol metabolizing enzymes. Among these studies, Kayaalti and Soylemezoglu [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

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

Reference	Population, (n)	Studied genes	Allele frequency, %
26	Healthy volunteers (n=211)	*ADH1B* *ALDH2*	*1=87.5%, *2=12.5% *1=100%, *2=0%
27	Healthy volunteers (n=88 for ADH1B, n=100 for ADH1C)	*ADH1B* *ADH1C*	*1=88.1%, *2=11.9% *1=31.5%, *2=68.5%
27	Alcoholics (n=73 for ADH1B, n=75 for ADH1C)	*ADH1B* *ADH1C*	*1=95.9%, *2=4.1% *1=26%, *2=74%
Present study	Healthy volunteers (n=81 for ADH1B and ALDH, n=80 for ADH1C)	*ADH1B* *ADH1C* *ALDH2*	*1=99.4%, *2=0,6% *1=45%, *2=55% *1=100%, *2=0%
Present study	Non-cirrhotic alcoholics (n=78 for ADH1B, n=73 for ADH1C)	*ADH1B* *ADH1C* *ALDH2*	*1=94.9%, *2=5.1% *1=36.3%, *2=63.7% *1=100%, *2=0%
Present study	Alcoholic cirrhotics (n=78 for ADH1B, n=68 for ADH1C)	*ADH1B* *ADH1C* *ALDH2*	*1=97.4%, *2=2.6% *1=47%, *2=53% *1=100%, *2=0%

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* genotype was more commonly seen 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 more frequent than the *ADH1C*1* allele in both alcoholic and healthy volunteers. Given that the *ADH1C*2* allele converts ethanol to acetaldehyde at a slower rate; it seems that Turkish people have a tendency for alcohol tolerance. On the other hand, the overall allele frequency of *ADH1C*1* and *ADH1C*2* was not statistically different between these two groups. Furthermore, genotype distribution and allele frequency of *ADH1C* was not statistically different between alcoholic cirrhotics and non-cirrhotic alcoholics. In this study, the *ALDH2*2* allele (inactive allele) was not observed in either control or alcoholic subjects. So, it can be concluded that the *ALDH2*2* inactive allele is not found in Turkish people like most of Caucasian race. The presence of the *ALDH2*1* allele or the absence of the *ALDH2*2* allele supports the data that the Turkish population is less susceptible to alcohol dependency.

CONCLUSION

In conclusion, allele **1* and **2* frequencies of the *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

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

[1] Mizoi Y, Yamamoto K, Ueno Y, Fukunaga T, Harada S. Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenase in individual variation of alcohol metabolism. Alcohol Alcohol 1994;29(6):707–710.

[2] Matsuo K, Wakai K, Hirose K, Ito H, Saito T, Tajima K. Alcohol dehydrogenase 2 His47Arg polymorphism influences drinking habit independently of aldehyde dehydrogenase 2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects. Cancer Epidemiol Biomarkers Prev 2006;15(5):1009–1013. http://dx.doi.org/10.1158/1055-9965.EPI-05-0911

[3] Osier M, Pakstis AJ, Kidd JR, Lee JF, Yin SJ, Ko HC, et al. Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism. Am J Hum Genet 1999;64(4):1147–1157. http://dx.doi.org/10.1086/302317

[4] Luo HR, Tu GC, Zhang YP. Detection of usual and uncommon aldehyde dehydrogenase alleles by mismatch amplification mutation assay. Clin Chem Lab Med 2001;39(12):1195–1197. http://dx.doi.org/10.1515/CCLM.2001.189

[5] Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PA, Richter MM, et al. Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet 2009;18(3):580–593. http://dx.doi.org/10.1093/hmg/ddn372

[6] Luo X, Kranzler HR, Zuo L, Wang S, Schork NJ, Gelernter J. Diplotype trend regression analysis of the ADH gene cluster and the ALDH2 gene: multiple significant associations with alcohol dependence. Am J Hum Genet 2006;78(6):973–987. http://dx.doi.org/10.1086/504113

[7] Roberts-Thomson IC, Butler WJ. Polymorphism and addiction to alcohol. J Gastroenterol Hepatol 2004;19(11):1322–1323. http://dx.doi.org/10.1111/j.1440-1746.2004.03631.x

[8] Terelius Y, Norsten-Höög C, Cronholm T, Ingelman-Sundberg M. Acetaldehyde as a substrate for ethanol-inducible cytochrome P450 (CYP2E1). Biochem Biophys Res Commun 1991;179(1):689–694. http://dx.doi.org/10.1016/0006-291X(91)91427-E

[9] Lieber CS. Ethanol metabolism, cirrhosis and alcoholism. Clin Chim Acta 1997;257(1):59–84. http://dx.doi.org/10.1016/S0009-8981(96)06434-0

[10] Iwahashi K, Ameno S, Ameno K, Okada N, Kinoshita H, Sakae Y, et al. Relationship between alcoholism and CYP2E1 C/D polymorphism. Neuropsychobiology 1998;38(4):218–221. http://dx.doi.org/10.1159/000026544

[11] Konishi T, Calvillo M, Leng AS, Feng J, Lee T, Lee H, et al. The ADH3*2 and CYP2E1 c2 alleles increase the risk of alcoholism in Mexican American men. Exp Mol Pathol 2003;74(2):183–189. http://dx.doi.org/10.1016/S0014-4800(03)00006-6

[12] Howard LA, Ahluwalia JS, Lin SK, Sellers EM, Tyndale RF. CYP2E1*1D regulatory polymorphism: association with alcohol and nicotine dependence. Pharmacogenetics 2003;13(6):321–328. http://dx.doi.org/10.1097/00008571-200306000-00003

[13] Teli MR, Day CP, Burt AD, Bennett MK, James OF. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. Lancet 1995;346(8981):987–990. http://dx.doi.org/10.1016/S0140-6736(95)91685-7

[14] Day CP, Bassendine MF. Genetic predisposition to alcoholic liver disease. Gut 1992;33(11):1444–1447. http://dx.doi.org/10.1136/gut.33.11.1444

[15] Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, et al. Distribution of ADH2 and ALDH2 genotypes in different populations. Hum Genet 1992;88(3):344–346. http://dx.doi.org/10.1007/BF00197271

[16] Chao YC, Liou SR, Chung YY, Tang HS, Hsu CT, Li TK, et al. Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. Hepatology 1994;19(2):360–366. http://dx.doi.org/10.1002/hep.1840190214

[17] Yamauchi M, Maezawa Y, Mizuhara Y, Ohata M, Hirakawa J, Nakajima H, et al. Polymorphisms in alcohol metabolizing enzyme genes and alcoholic cirrhosis in Japanese patients: a multivariate analysis. Hepatology 1995;22(4):1136–1142.

[18] Day CP, Bashir R, James OF, Bassendine MF, Crabb DW, Thomasson HR, 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(5):798–801. http://dx.doi.org/10.1002/hep.1840140509

[19] Chen CC, Lu RB, Chen YC, Wang MF, Chang YC, Li TK, et al. Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. Am J Hum Genet 1999;65(3):795–807. http://dx.doi.org/10.1086/302540

[20] 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 1995;23(5):519–523. http://dx.doi.org/10.1016/0168-8278(95)80056-5

[21] Zintzaras E, Stefanidis I, Santos M, Vidal F. Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? Hepatology 2006;43(2):352–361. http://dx.doi.org/10.1002/hep.21023

[22] Day CP, James OF, Bassendine MF, Crabb DW, Li TK. Alcohol dehydrogenase polymorphisms and predisposition to alcoholic cirrhosis. Hepatology 1993;18(1):230–232. http://dx.doi.org/10.1002/hep.1840180140

[23] Chinnaswamy P, Vijayalakshmi V. Subtypes of ADH2 gene in alcoholics. Indian J Clin Biochem 2005;20(2):104–109. http://dx.doi.org/10.1007/BF02867408

[24] Vidal F, Lorenzo A, Auguet T, Olona M, Broch M, Gutiérrez C, et al. 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. J Hepatol 2004;41(5):744–750. http://dx.doi.org/10.1016/j.jhep.2003.06.003

[25] Tamakoshi A, Hamajima N, Kawase H, Wakai K, Katsuda N, Saito T, et al. Duplex polymerase chain reaction with confronting two-pair primers (PCR-CTPP) for genotyping alcohol dehydrogenase beta subunit (ADH2) and aldehyde dehydrogenase 2 (ALDH2). Alcohol Alcohol 2003;38(5):407–410. http://dx.doi.org/10.1093/alcalc/agg096

[26] Kayaaltı Z, Söylemezoğlu T. Distribution of ADH1B, ALDH2, CYP2E1 *6, and CYP2E1 *7B genotypes in Turkish population. Alcohol 2010;44(5):415–423. http://dx.doi.org/10.1016/j.alcohol.2010.06.002

[27] Aktas EO, Kocak A, Senol E, Celik HA, Coskunol H, Berdeli A, et al. Determination of the effects of alcohol dehydrogenase (ADH) 1B and ADH1C polymorphisms on alcohol dependence in Turkey. Sci Justice 2012;52(1):58–61. http://dx.doi.org/10.1016/j.scijus.2011.05.002