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ALLELIC VARIANTS AT ALCOHOL METABOLISING GENES IN TURKISH POPULATION

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SUMMARY

Alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) play central roles in the initial stages of alcohol metabolism. Polymorphisms with physiological significance exist in the *ADH* and *ALDH* genes to different degrees in different ethnic groups. Specifically, *ADH1B*2 (ADH1B**47His), *ADH1B*3* (*ADH1B**369His), *ADH1C*1* (*ADH1C**349Ile) and *ALDH2*2 (ALDH2**487Lys) variants have been shown to confer protection against alcohol toxicity and dependence. During the past decade, the prevalence of alcoholism in Turkey has significantly increased with the economic development and industrialization of the country. Thus, the aim of this study was to investigate genetic differences in polymorphisms of alcohol-metabolizing enzymes, *ADH1B, ADH1C* and *ALDH2,* for Turkish population by using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. Also, the frequencies of alleles were compared with those obtained from different populations. In conclusion; the allelic frequencies for *ADH1B*2, ADH1B*3, ADH1C*1* and *ALDH2*2* were observed 0.049, 0.004, 0.397 and 0.018, respectively. Turkish people resemble Caucasians while being different from Asians in terms of the distribution of *ADH1B*2, ADH1C*1*, *ALDH2*2* alleles. Furthermore, Turkish people have less *ADH1B*3* allele than the other populations, especially African-Americans.

Key words: ADH, ALDH, Turkish population, Genotype frequency

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INTRODUCTION

Alcohol is metabolised by *ADH* into acetaldehyde, which is further converted by *ALDH* into acetate. The alcohol metabolising enzymes exhibit genetic polymorphisms that results in different levels of expression and activity. The genes coding these enzymes are likely to contribute variations in alcohol metabolism, and individual vulnerability for developing alcohol dependence and alcohol-related disabilities (1).

Two functional SNPs in *ADH1B* are a 175A/G substitution in exon 3 (dbSNP rs1229984, resulting in the substitution of arginine 47 by histidine and referred to as *ADH1B*2*) and a 1139C/T substitution in exon 9 (dbSNP rs2066702, resulting in the substitution of arginine 369 by cysteine and referred to as *ADH1B*3*). Both alleles are associated with higher *ADH1B* activity compared to *ADH1B*1*. The other functional *ADH* variant is *ADH1C*, its polymorphism is due to a 1119A/G substitution in exon 8 (dbSNP rs698, resulting in the substitution of isoleucine 349 by valine and is referred to as *ADH1C*2*). *ADH1C*2* allele is associated with reduced *ADH1C* enzyme activity compared to the wild type *ADH1C*1* allele. The allelic variants *ADH1B*2*, *ADH1B*3* and *ADH1C*1* result in enzymes with increased activity and therefore increased capacity to convert alcohol to the carcinogenic and unpleasant acetaldehyde, the accumulation of which is associated with increased alcohol sensitivity and leading to a general intolerance to alcohol (2, 3).

Only one functional SNP in *ALDH2*, a 1543G/A substitution (dbSNP rs671, resulting in the substitution of glutamate 487 by lysine and is referred to as *ALDH2*2*) is associated with reduced *ALDH2* activity. *ALDH2*2* allele results in reduced clearance of acetaldehyde and has been associated with protection against alcohol toxicity and alcohol dependence because of the unpleasant side effects of the accumulated acetaldehyde (3).

The aim of the study was to predict the individual vulnerability of Turkish population to develop alcohol dependence and alcohol-related disabilities and to compare the genotype frequencies of this population with those of other world populations for the *ADH1B*2, ADH1B*3*, *ADH1C*1*, *ALDH2*2* alleles reported as being protective against alcohol in several studies.

MATERIALS AND METHODS

Sample collection; Following the approval of the Ethics Committee of Istanbul University, blood samples were collected from 122 healthy volunteers in Turkish population. The average age of these subjects was 32.09 (±10.3) and 41.7% of healthy volunteers were male. Histories of the volunteers revealed no consumption of alcohol or chronic disease, no evidence of liver disease at physical examination, and had normal liver function test results. The geographical distributions of all subjects were from all regions of the country representing the Turkish population.

PCR-RFLP methods; Genomic DNA was extracted from blood samples using a sodium perchlorate/chloroform extraction (4). Genotyping of *ADH1B* (Arg47His and Arg369Cys), *ADH1C* (Ile349Val) and *ALDH2* (Glu487Lys) variants was performed by PCR-RFLP methods. PCR were amplified from genomic DNA in the presence of specific primers (Table 1). For amplification, primers and annealing temperatures were shown in Table 1. The PCR products were digested with the various restriction enzymes. The used restriction enzymes and the obtained DNA bands, belonging to the product size, were shown in Table 1. To assess reliability of genotyping, analysis was repeated on 10% of the samples and DNA sequencing was performed on representative samples for all genotypes and SNPs.

Table 1: Primers, restriction enzymes, and results for *ADH* and *ALDH* variations.

SNP	Sequences of forward and reverse primers	T° Anneal	Restriction enzyme, Length of RFLP products (bp)*
ADH1B Arg47His rs2066702	5′-TTCTGTAGATGGTGGCTGTA-3′ 5′- GAAAGAGGAAACTCCTGAAG-3′	59 °C	MsII, 287, 253, 34
ADH1B Arg369Cys rs1229984	5′-GATGAACTTCCTTTTTCTTG-3′ 5′-CTCTGAAGAGCTGAATTAAT-3′	54.5 °C	AlwNI, 288, 166, 122
ADH1C Ile349Val rs698	5′-TGGTTGAATCTATCAATGAT-3′ 5′-CACTGTAATTTTTTCTCATC-3′	53 °C	SspI, 290, 171, 119
ALDH2 Glu487Lys rs671	5′-GATGTGTTTGGAGCCCAGTC-3′ 5′-TAAATCCCTGGAAGGCCCAG-3′	58 °C	MboII, 135, 126, 9

*bp, base pair

Statistical analysis; The genotype and allele frequencies were determined by direct counting. Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium. The Hardy-Weinberg equilibrium was tested for all polymorphisms. Differences in allele frequencies between other populations in the world were determined using Fisher's exact test with two-tailed *p* values. A *p* value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A number of studies have looked for polymorphic variants of *ADH* and *ALDH* in alcohol metabolizing enzymes to explain the differences in responses to alcoholism and individual vulnerability for developing alcohol dependence and alcohol-related disabilities. Differences in alcohol metabolism that result from these enzyme polymorphisms could also affect cancer etiology among drinkers (5-8).

ADH and *ALDH* exist as multiple isozymes that differ in their kinetic properties. *ADH1B*1/*1* allele has only 1% and 0.5% of the oxidation capability of *ADH1B*1/*2* and *ADH1B*2/*2* alleles, respectively (9). *ADH1B*1* allele is barely active in the metabolism of alcohol, while *ADH1B*3* allele is highly active (10). *ADH1C*1* and *ADH1B*2* alleles code "fast" metabolism of alcohol. *ADH1C*1* allele metabolises alcohol into acetaldehyde 2.5 times faster than *ADH1C*2* allele (2). Besides, linkage disequilibrium between *ADH1C*1* and *ADH1B*2* alleles has been demonstrated in several Caucasian populations (11). It was reported that *ADH1B* allele frequencies differ between alcoholics and non-alcoholics, with a higher occurrence of *ADH1B*2* allele in non-alcoholics (12-16) and in moderate drinkers relative to heavy drinkers (Neumark *et al*., 2004). *ADH1B*3* allele is associated with lower rates of heavy drinking and alcohol dependence in native Americans (17) and with a negative family history of alcoholism in African-Americans (1). The effects of the *ADH1C*1* allele are considerably smaller than those of *ADH1B*2* allele (16). While no association is usually found between *ADH1C* variations and alcoholism incidence in European populations (12, 18), this allele is suggested to provide a protection against alcohol abuse and alcoholism, as demonstrated by a higher frequency of this allele in non-alcoholics, especially in Asian populations (14, 19). As to *ALDH2*, the alleles that encode the active and inactive subunits are *ALDH2*1* and *ALDH2*2*, respectively. Individuals who are *ALDH2*2* homozygous have

null *ALDH2* activity, and those who are heterozygous have approximately 6% residual activity; both groups are characterised by an *ALDH2*-deficient phenotype (20). This leads homozygous and heterozygous possessors of the *ALDH2*2* allele to experience a build-up of acetaldehyde that creates a toxic reaction, including flushing, increased heart rate, and nausea (9). By Thomasson *et al*. (15), it has been calculated that the *ALDH2*2* allele provides a 10-fold reduction in the risk of alcohol dependence. Individuals carrying the *ALDH2*2* allele drink less alcohol (21, 22) and have a lower prevalence of binge drinking (22).

ADH and *ALDH* genes vary markedly in frequency among different ethnic and racial groups. Even though it has been researched on different populations, there are only two studies on the frequencies of *ADH* and *ALDH* genes in Turkish population (23, 24). In these studies, *ADH1B* Arg47His, *ADH1B* Arg369Cys and *ALDH2* Glu487Lys were studied. However, there was no data on *ADH1B*3,* highly active allele, in Turkish population. In the present study, four SNPs, the most responsible enzyme variants in the metabolism of alcohol, were genotyped together in Turkish population (Table 2). Besides, we compared the genotype frequencies of this population with those of other world populations (Table 3).

Table 2: Genotype distributions of *ADH* and *ALDH* variants in the studied Turkish population.

Genotype Distrubition		
***ADH1B* Arg47His**		
G/G	G/A	A/A
(Arg/Arg)	(Arg/His)	(His/His)
110	12	0
***ADH1B* Arg369Cys**		
C/C	C/T	T/T
(Arg/Arg)	(Arg/Cys)	(Cys/Cys)
121	1	0
***ADH1C* Ile349Val**		
G/G	G/A	A/A
(Ile/Ile)	(Ile/Val)	(Val/Val)
21	55	46
***ALDH1C* Glu487Lys**		
G/G	G/A	A/A
(Glu/Glu)	(Glu/Lys)	(Lys/Lys)
119	3	0

Table 3: Comparison the allele frequencies of *ADH* and *ALDH* genes in Turkish population obtained from the present study with those in the other populations reported by the previous studies.

Populations		Frequency		
		ADH1B*2 **(*ADH1B**47His)**	***ADH1C*1*** **(*ADH1C**349Val)**	***ALDH2*2*** **(*ALDH2**487Lys)**
Turkish Population				
	The present study (n=122)	**0.049**	**0.397**	**0.018**
	Kortunay et al. (23) (n=102)	–	0.660	–
	Kayaalti et al. (24) (n=211)	0.080	–	NO
Caucasians				
	Goedde et al. (25) (n=117)	0.104	–	0.013
	Garcia-Martin et al. (26) (n=255)	0.077	–	–
	Tiemersma et al. (27) (n=869)	–	0.817	–
	Chichoz-Lach et al. (28) (n=198)	–	0.528	–
	Barras et al. (12) (n=876)	0.201	0.570	–
	Chichoz-Lach et al. (10) (n=342)	0.020	0.571	–
Asians				
	Yang et al. (29) (n=389)	0.607	–	0.267
	Tseng et al. (30) (n=198)	0.550	–	0.194
	Shen et al. (14) (n=339)	0.572	0.873	0.118
	Chen et al. (19) (n=885)	0.629	0.876	0.181
	Hasegawa et al. (31) (n=1585)	–	–	0.154
Americans				
	Konishi et al. (32) (n=104)	0.043	–	NO
	Loza et al. (33) (n=118)	0.090	–	NO
	Mulligan et al. (34) (n=582)	NO	0.600	–
	Gordilla-Bastidas et al. (35) (n=218)	0.034	–	NO
Africans				
	Goedde et al. (25) (n=49)	NO	–	NO

NO: No observed

*ADH1B*1* is the predominant allele in most populations. However, *ADH1B*2* allele is common in Asian populations in contrast to the other population (Table 3). In Turkish population, there are only one study on the distribution of *ADH1B*2* alleles (23). Similar to the results reported by Kayaalti *et al*. (24), we found the frequency of *ADH1B**2 were 0.049. When compared the *ADH1B**2 allele frequency in Turkish populations with in the other population, Caucasians and Americans had similar allelic frequencies. *ADH1B*3* was no observed in African populations by Goedde *et al.* (25). However, the Caucasians population studied from Barras *et al.* (12) had much greater relative allele frequency in contrast with the studied Turkish population ($p<0.002$). For genotyping, the authors studied 876 white individuals from the following populations; Tarragona (Spain), Bor-

deaux (France), Heidelberg (Germany), Stockholm (Sweden), and Krakow (Poland) (Table 3).

*ADH1B*3* allele occurs in 25% of the African-Americans, i.e. relatively more often than among white Americans, Caucasians and Asians (11, 36). Our results showed that no individuals had *ADH1B*3* 369Cys allele and all individual had *ADH1B*3* 369Arg except for only one person who had *ADH1B*3* heterozygote (Table 2). Turkish people resemble Caucasians and Asians in terms of the distribution of *ADH1B*3* allele (11, 36).

In Caucasians, 45–70% are heterozygous *ADH1C*1/*2*. By contrast, the frequency of the *ADH1C*1* allele is 75–90% in Africans and 85–100% in Asian populations (9, 37) (Table 3). Kortunay *et al.* (23) performed the genotyping of *ADH1C*1* in 102 healthy Turkish people. They found the frequency of *ADH1C*1* allele to be 0.66, which was higher than the frequency of our population (Table 3). Compared the studied population with the other world populations, our results showed that Turkish population had significantly less *ADH1C*1* allele ($p<0.02$) than the other populations (%40). Besides, Caucasian population (n=869) studied by Tiemersma *et al.* (27) had more *ADH1C*1* allele frequency in contrast with the studied Turkish population and the other studied Caucasian populations ($p\leq0.001$) (Table 3).

*ALDH2*2* mutant allele is prevalent in Asians, with a frequency of up to 40%, whereas it does not exceed 5% in Caucasians and African populations (9). In Asians, *ALDH2*2* allele frequency was high in comparison with the studied Turkish Population. However, no significant difference was observed in our population when compared with Caucasians (25) (Table 3).

In conclusion; the present study could be useful in understanding the allele distributions of alcohol metabolizing enzymes, *ADH* and *ALDH*, in Turkish population because these alleles, which can be observed on various races and even on sub-populations, have not been sufficiently researched in Turkish population. Turkish people resemble Caucasians while being different from Asians in terms of the distribution of *ADH1B*2, ADH1C*1*, *ALDH2*2* alleles and from African-Americans in terms of distribution of *ADH1B*3*. Moreover, knowledge obtained from the present study might be useful in future association studies assessing sensitivity to cancer or the other disabilities related to alcohol consumption and alcohol dependence. According to our results, it can be suggested the studied Turkish population may have the risk of developing alcoholism.

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