Alcohol dehydrogenase genotype (ADH2 and ADH3) in alcoholism of Iraqi people

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Objective The polymorphic human liver enzyme alcohol dehydrogenase (ADH) is responsible for the oxidative metabolism of ethanol. An allele encoding active form of cytosolic enzyme is known to reduce the likelihood of alcoholism in Iraqi men. The polymorphisms of ADH modifies the predisposition to the development of alcoholism. Determination of genotypes ADH2 and ADH3 loci in alcoholic and non-alcoholic Iraqi men.

Method Using leukocyte DNA extraction and then amplifying by polymerase chain reaction (PCR) by using specified primers, then allele specific primer extension and PCR-restriction fragment length polymorphism (RFLP) methods with another set of primers is employed in order to determine the variants of ADH2 and ADH3, respectively.

Results The Iraqi alcoholics had significantly lower frequencies of ADH2*2 and ADH3*1 alleles than did the non-alcoholic as compared with the general population of East Asians but more than in Caucasians population, suggesting that genetic variation in ADH enzyme modulating the rate of metabolism of ethanol to acetaldehyde influences drinking and the risk of developing alcoholism. The simplest explanation of the significant lower frequency of ADH2*2 and ADH3*1 alleles among Iraqi alcoholic men is that each can produce higher transient level of acetaldehyde, which trigger aversive reactions; these alleles are less likely to become alcoholic.

Conclusion This study suggests that both ADH2 and ADH3 genotypes exert an influence on alcohol metabolic rate, alcohol-flush reaction and susceptibility to develop alcoholism. ADH2 and ADH3 genotypes may have a protective role in the risk for alcoholism in Iraqi alcoholic population.

Keywords alcohol dehydrogenase, genotype, alcoholism, cytosolic enzyme

Introduction

Ethanol is a kind of drug affecting the mind, mood and other mental processes. Consumption of ethanol is very common throughout the world. Uncontrolled or excessive intake of alcoholic beverages is defined as alcoholism. Alcoholism is a public concern for many countries, because it affects physical or mental health, social and familial relationships, and occupational responsibilities.[1] Individuals give different responses when exposed to comparable amount of alcohol. Alcoholism must be accepted as a chronic disorder with a complex origin and outcome.[2]

Alcoholism is an important cause of chronic liver diseases, but only 10%–20% of alcoholics develop cirrhosis.[3] While a group of alcoholics do not develop cirrhosis or other chronic liver diseases, other groups who possibly consume less alcohol can have considerable liver damage. Individual-based genetic variations in the genes encoding the enzymes playing active role in ethanol metabolism are considered to be responsible for this difference.[4]

Most ethanol elimination occurs by oxidation to acetaldehyde and acetate, catalyzed principally by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). There are multiple isozymes of ADH in human liver. The ADH isozymes primarily involve in hepatic ethanol metabolism are homo and hetero dimeric molecules whose subunits are encoded by ADH1, ADH2 and ADH3 genes[5] and are closely linked to chromosome 4.[6,7]

Polymorphic alleles at the ADH2 (B-subunit) and ADH3 (γ-subunit) loci encode isozymes that differ strikingly in catalytic properties[5] These differences are thought to underlie apart of the threefold variation in alcohol elimination rates among individuals (Wagner et al, 1976) of which 50% is thought to be genetic in origin.[8,9]

Humans have seven ADH genes tightly clustered on chromosome 4q22 in a head-to-tail array extending over 365 kb. The order of the genes (from 5' to 3') is ADH7-ADH1C- ADH1B-ADH1A-ADH6-ADH4-ADH5, running as shown in Fig. 1.[10,11]

All the ADH enzymes are broad substrate oxidoreductases that use NAD$^+$/NADH as cofactors.[10] ADH1A, ADH1B and ADH1C encode α, β and γ subunits, respectively; these can form heterodimers, and are defined as class I ADH forms. Class I ADHs have K_m value for ethanol ranged between 0.05–34 mM.[10] ADH4 encodes π-ADH, a class II ADH with K_m value for ethanol of 34.0 mM. ADH5 encodes x-ADH, which is also a glutathione-dependent formaldehyde dehydrogenase, χ-ADH has very low affinity for ethanol. ADH7 encodes σ-ADH (also known as μ-ADH); it is the most efficient of these enzymes at oxidising retinol. The protein encoded by ADH6 has not been purified from tissue (Table 1).

The primary site of ethanol oxidation is the liver, in which there are high concentrations of most of the ADHs, except σ-ADH.[10] Consideration of the enzyme concentrations in liver and the kinetic properties of the ADHs suggest that class I enzymes (encoded by ADH1A, ADH1B and ADH1C) and class II enzyme (encoded by ADH4) make the most significant contribution to ethanol metabolism.[10,12] (Hurley, 2002 ; Lee, 2004). It has been calculated that class I enzyme contributes ~70% of the total ethanol oxidising capacity of the liver at an ethanol concentration of 22 mM (0.1%; 0.08% is defined as

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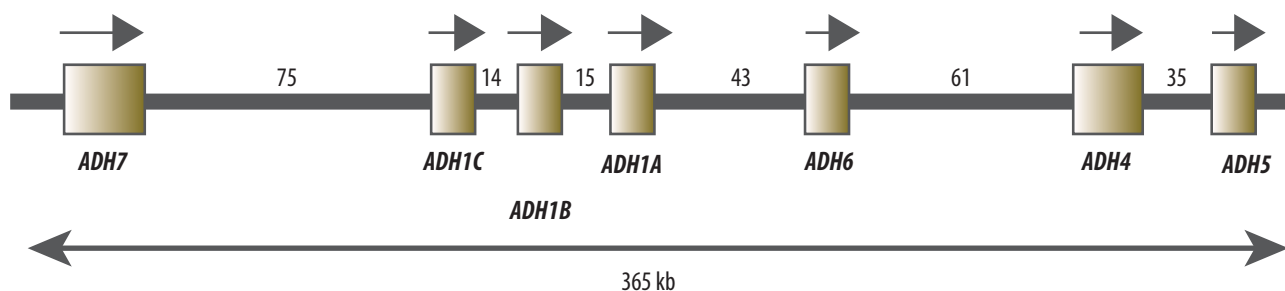
Fig. 1 **Relative sizes and position of the seven human ADH gene on the long arm of chromosome 4 (i.e. chromosome 4q).**

Table 1. **ADH genes and proteins**[10]

Official gene name*	Old name†	Non-standard name‡	Protein	Class¶
ADH1A	ADH1	ADH1A	A	I
ADH1B	ADH2	ADH1B	B	I
ADH1C	ADH3	ADH1C	Γ	I
ADH4	ADH4	ADH2	Π	II
ADH5	ADH5	ADH3	X	III
ADH6	ADH6	ADH5	ADH6	V
ADH7	ADH7	ADH4	Σ	IV

ADH: alcohol dehydrogenase.

Table 2. **Kinetic properties of ADH proteins**

Official gene name*	Amino acid differences between alleles	Protein name	K_m (ethanol) mM	Turnover (min⁻¹)
ADH1A		A	4.0	30
ADH1B*1	Arg48, Arg370	β_1	0.05	4.0
ADH1B*2	His48, Arg370	β_2	0.9	350
ADH1B*3	Arg48, Cys370	β_3	40	300
ADH1C*1	Arg272, Ile350	γ_1	1.0	90
ADH1C*2	Gln272, Val350	γ_2	0.6	40
ADH1C*352Thr	Thr 352†	----	----	----
ADH4		Π	30	20
ADH5		X	>1,000	100
ADH6		ADH6	?	?
ADH7		Σ	30	1800

ADH: alcohol dehydrogenase.

legally intoxicated in Iraq), and class II enzyme contributes ~30% (Table 2).[10,12]

The pharmacokinetics of ethanol metabolism influences the risk for alcohol dependence. Various studies have shown that coding variations in the genes encoding two alcohol-metabolising enzymes, ADH1B and ADH1C are associated with risk for alcoholism.[10,13] The ADH1B*2 allele in which arginine 48 is replaced with histidine encodes the B2 subunit, which has a 40-fold higher V_{max} than the B1 subunit encoded by ADH1B1 (48 arg370 reference allele).[12] ADH1B*2 is relatively common among Asians, where it has been shown to be protective against

alcoholism[10,14]; although rarer in Europeans, it has also been shown to be protective in that group.[15,16] A different allele, ADH1B*3, encodes the B3 subunit in which arginine 370 is replaced by cysteine; the B3 subunit has a 30-fold higher V_{max} than the B1 subunit. ADH1B*3 is relatively common among individuals of African ancestry, and individuals carrying this polymorphism have a higher rate of metabolising alcohol.[17] ADH1C, which encodes the γ subunit, has polymorphisms at amino acids 272 and 350; these are in high linkage dis-equilibrium (LD), with the 272Arg–350 Ile form called γ1 (encoded by ADH1C1) and the 272Gln–350Val called γ2 (encoded

by ADH1C*2) (Hoog,1986).[18] The V_{max} of γ1 is about twice that of γ2.[10,12,19]

The aim of the present study is to identify the ADH variants in alcoholic and non-alcoholic Iraqi people, and also to investigate the relationship between ADH gene polymorphism with the tendency of Iraqi people to develop alcohol tolerance, and then to compare with other ethnic groups and races in world.

Materials and Methods

Seventy alcoholic male Iraqi people with the mean ± SD (40.35 ± 11.01 years old) who were alcohol dependent by DSM-III criteria (American Psychiatric Association 1980),[20] were selected from Ibn-Rushed Teaching Hospital/Baghdad, Iraq from Jan. to Sep. 2013. Another 70 non-alcoholic subjects were selected as control group from the male students, hospital staff (physicians, laboratory staffs, pharmacists and others) with mean ± SD (33.02 ± 7.83 years old). Informed consent was obtained, and 10 ml of blood samples was drawn from each subject. Genomic DNA kit from BIONEER, Inc. Company, South Korea, was used to extract DNA and then polymerase chain reaction (PCR) was performed to amplify target DNA.

Genotyping of ADH3

We chose two primers (5'-AATC-TACCTCTTTCCAGAGC-3') and (5'-GC TTTAAGAGTAAATAATCGTCC CC-3') to amplify a fragment of 146 bp for alleles detection, aliquots of amplified DNA products were digested with Ssp1 (restriction enzyme) at 37°C for 16 hours (overnight). Ssp1 was provided by Takara Bio Inc., Japan.

Genotyping of ADH2

Allele-specific primer extension – PCR method was chosen, ADH2 gene located in 4q22 has a functional polymorphism Arg48His. Two pairs of allele specific primers were used for ADH2 genotyping.
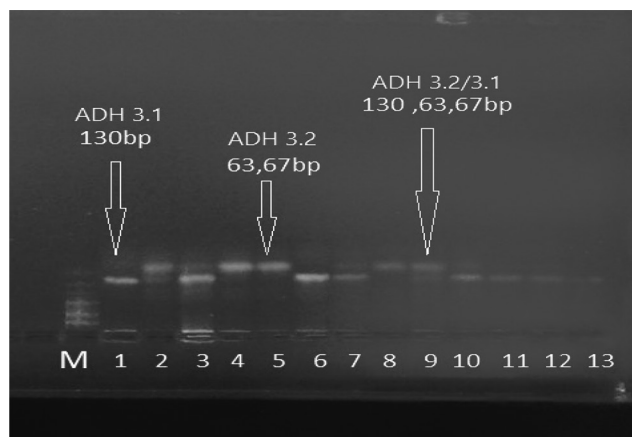
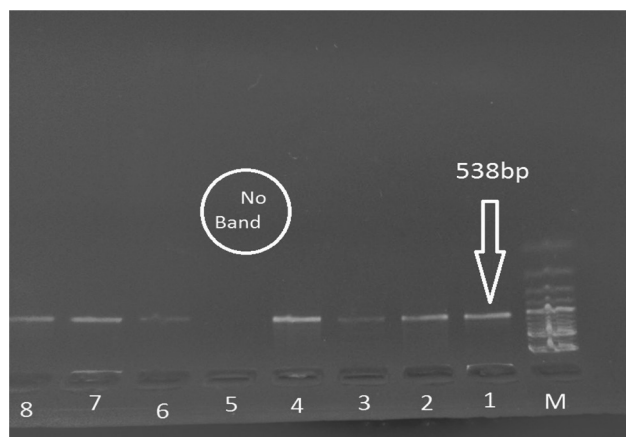Fig. 2 **Screening of ADH3 genotyping on Agarose gel electrophoresis.**

Fig. 3 **Screening of ADH2 genotyping on Agarose gel electrophoresis.**

Table 3. **Distribution of ADH2 genotypes for both alcoholics and control group**

Gene	No.	ADH2.1	ADH2.2	Allele1*	Allele2*
ADH2 (alcoholics)	70	47(67%)	23(33%)	62	38
ADH2 (Control)	70	35(50%)	35(50%)	50	50
P value		0.05	0.075	0.049	0.06

Table 4. **Distribution of ADH3 genotypes for both alcoholics and control group**

Gene	No.	ADH3.1	ADH3.2	ADH3.1/3.2	Allele1*	Allele2*
ADH3 (alcoholics)	70	17 (24%)	23 (33%)	30 (43%)	40.5	59.5
ADH3 (control)	70	26 (37%)	18(26%)	26 (37%)	50	50
P value		0.082	0.041	0.002	0.03	0.036

- For 48 Arg allele: ADH 2.1, 538 bp.
Forward Primer: (5'-TCTGTAGATGGT-GGCTGTAGGAATCTGAC G-3'). This primer is arginine specific (CGC). The third base from 3'-end is changed from T→A. This primer binds on Exon III.
Reverse Primer: (5'-TACTTTTTTTCCC TCCTCCCGTTTCTACTTCTA-3'). This is sequence specific primer binding on Intron III.
- For 48 His allele: ADH 2.2, 538 bp.
Forward Primer: (5'-TCTGTAGATGGT-GGCTGTAGGAATCT GCCA-3'). This primer is histidine specific (CAC). The third base from 3'-end is changed from T→C. This binds on Exon III.
Reverse Primer: (5'-TACTTTTTTTCCCT CCTCCCGTTTCTACTTCTA-3'). This is sequence specific primer binding on Intron III.[1]

PCR primers were provided by Bioneer Inc. Company.

Results

Both the PCR products were evaluated on 2% of agarose gel electrophoresis.

The three alleles included in ADH3 variant which appear in the regions 63 and 67 bp (polymorphic type) which is also known as ADH3.2, 146 bp (wild type) (ADH3.1) and (63,67,146) (heterozygosity) (ADH3.1/3.2) were detected as shown in Fig. 2.

For ADH2 variant, two alleles appear, one in the region 538bp (ADH2*1) and no band for ADH2*2 allele, see Fig. 3.

We use two statistical analysis programs in this study, SPSS-IBM and MINITAB 17 to compare between groups.

There were striking differences between the alcoholics and the non-alcoholics in both the genotype and allele frequencies, at two loci examined see (Tables 3 and 4).

The ADH2*2 and ADH3*1 alleles were all significantly less frequent among alcoholics than among non-alcoholics. Both of these alleles encode the higher activity forms of B2 and γ1, respectively.

The allele ratio ADH3*2/ADH3*1 is most common in alcoholic and non-alcoholic subjects (P = 0.002).

ADH3*2 and ADH2*1 were higher in alcoholic than in non-alcoholic, both of these alleles encoded the low activity forms.

The difference in ADH2 and ADH3 allele frequencies was still significant between the two groups. It appears that theADH2*2 and the ADH3*1 alleles are protective against the development of alcoholism.

Discussion

The present article is the first report of a significant difference in ADH2 and ADH3 genotypes between alcoholic and non-alcoholic Iraqi men. Alcoholics have significantly lower frequencies of both ADH2*2 and ADH3*1 alleles than that found in non-alcoholics from the same population (Table 4). This indicates that the ADH2 and ADH3 alleles affect the propensity for alcoholism.

ADH2 and ADH3 are closely linked[7] to chromosome 4 (Tsukahara and Yoshida 1989). Among the alcoholics homozygous for ADH2*2, the ADH3*1 allele frequency is significantly different than that among the total population of non-alcoholics. Among the alcoholics homozygous for ADH2*1 and ADH3*2, allele frequency is significantly higher (P < 0.041) than that found in the non-alcoholic population. Thus, theADH3*2 allele appears to be accompanying the ADH2*1 allele.

From Table 2, we conclude that individuals possessing ADH2*2 and ADH3*1 alleles should, therefore, generate acetaldehyde more rapidly after ethanol consumption than do individuals with only ADH2*1 and ADH3*2 alleles. As they do not experience the side effects of acetaldehyde, they

Table 5. **Distribution of ADH2 and ADH3 genotypes in Caucasian race and Oriental people**

Genotype	Distribution in Caucasian race (%)	Distribution in Oriental people (%)	Results of the present work (%)
ADH3.1	50–60	90	40
ADH3.2	40–50	10	59
ADH2. 1	90	30	62
ADH2.2	0–10	70	38

consume more ethanol. The simplest explanation of the significantly lower frequency of ADH2*2 and ADH3*1 alleles among alcoholic men in Iraqi is that each can produce higher transient levels of acetaldehyde, through faster production, and that even transient elevation of acetaldehyde may trigger aversive reactions. These aversive reactions may make people with these alleles less likely to become alcoholics.

Our results have suggested that ADH2*2 has the major effects on alcoholics than the minor effects obtained from ADH3*1.

Table 5 shows that the distribution of ADH2 and ADH3 genotypes in Caucasian race are strongly different from Oriental people. ADH2*2 and ADH3*1 were high in Orient people and low in Caucasian while ADH3*2 and ADH2*1 were low in Orient people and high in Caucasian. Our findings lie in between them, which suggested that Iraqi people have a considerable chance to avoid or become alcohol tolerance.

Conclusion

1. This study suggests that both ADH2 and ADH3 genotypes exert an influence on alcohol metabolic rate, alcohol-flush reaction and susceptibility to develop alcoholism. ADH2 and ADH3 genotypes may have protective roles in the risk for alcoholism in Iraqi alcoholic population.

2. Higher acetaldehyde generated by more active ADH isozymes should deter heavy drinkers. Since the kinetic differences among ADH2 encoded B isozymes are much more striking than those between ADH3 encoded γ1 isozymes. We expected that the differences arising from the ADH2 alleles play larger role in affecting the risk of alcoholism. The simplest explanation of the significant lower frequency of ADH2*2 and ADH3*1 alleles among Iraqi alcoholic men is that each can produce higher transient level of acetaldehyde, which trigger a aversive reactions; these alleles are less likely to become alcoholic. ◼

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