

Single Nucleotide Polymorphisms of the Alcohol Dehydrogenase Genes among the 28 Caste and Tribal Populations of India

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ABSTRACT We report single nucleotide polymorphisms (SNPs) at the four sites in ADH2 and ADH3 genes among the 28 populations from southern parts of Andhra Pradesh, India. A total of 1048 individuals belonging to 28 endogamous populations distributed in the contiguous areas of the 6 southernmost districts of Andhra Pradesh were enrolled for the present study. Genotyping involved PCR and sequencing. We sequenced exon 3 and 9 of *ADH2* and exon 8 of *ADH3*, besides the ADH2 3'UTR- rs17033 (72 bases down stream of ADH2 Arg369Cys). The two sites of ADH2 (Arg47His and Arg369Cys) are found to be completely monomorphic showing only Arg47 and 369Arg (ADH2*1 allele), the remaining two sites were polymorphic. None of the 28 populations of this study deviated significantly from Hardy Weinberg Equilibrium proportions. The allele frequencies do not show any clear trend across socioeconomic groups. The degree of heterogeneity in the genotype frequencies among the hierarchical groups is significant for Ile349Val (df = 12; $\chi^2 = 22.050$) and not for the 3'UTR rs17033 (df = 6; $\chi^2 = 9.765$). The haplotype distribution among the hierarchical groups is found to be highly homogeneous and statistically nonsignificant ($\chi^2 = 0.248$, df = 18). Linkage disequilibrium does not exist between the two-polymorphic loci. The results were interpreted in the light of cultural patterns of the Indian hierarchical society.

INTRODUCTION

Alcohol dehydrogenase (ADH), a rate-limiting enzyme in alcohol metabolism, catalyzes the oxidation of ethanol to acetaldehyde. Alcohol dehydrogenase isoenzymes found in humans are unique, when compared with other animals. Seven ADH genes have been identified, characterized, and mapped to a gene cluster, located on chromosome 4 (Edenberg 2000). The different ADH isoenzymes have been classified based on enzymatic and DNA/protein sequence characteristics (Duester et al. 1999; Edenberg 2000). Genes for class I and IV ethanol oxidizing ADH have been well studied. The human system has additional complexity due to a gene triplcation and polymorphism that occurs among the class I isoenzymes, giving rise to the following class I genes: *ADH1*, *ADH2*1*, *ADH2*2*, *ADH2*3*, *ADH3*1*, and *ADH3*2*. The protein products of these genes, previously named α , β 1, β 2, β 3, γ 1 and γ 2, can form random associations to yield both homo and hetero-

dimeric forms (Edenberg and Bosron 1997). These genes are approximately 15 kb in size with nine exons (Duester et al. 1986). No polymorphisms that affect enzyme activity have been described for the ADH1 gene, but the effects of diallelic amino acid substitutions within each of the ADH2 and ADH3 genes upon *in vitro* enzyme kinetics are well documented (Bosron et al. 1983; Edenberg and Bosron 1997). The ADH2*2 allele, consisting of the atypical β 2 subunit, exhibits about 100 times more catalytic activity for ethanol oxidation than the usual ADH2*1/*1 allele at physiological pH (Yoshida 1983). The ADH3*1 allele with a γ 1 subunit has a V_{max} about twice that of γ 2 encoded by ADH3*2 (Bosron et al. 1988). The kinetic properties of these genes led to a hypothesis for an etiological role in the development of problems associated with alcohol consumption (Shen et al. 1997; Whitfield 1997, Whitfield et al. 1998; Chen et al. 1999; Osier et al. 1999) because of their role in the metabolism of ingested alcohol.

A substantial variation in the frequency of allele *ADH2*2* (*ADH2*47His*), which protects from alcoholism, has been reported among the different ethnic groups. This allele codes for a protein with substitution Arg47 to His and, consequently, results in higher enzymatic activity (Osier et al. 2002; Whiteld 2002). ADH2

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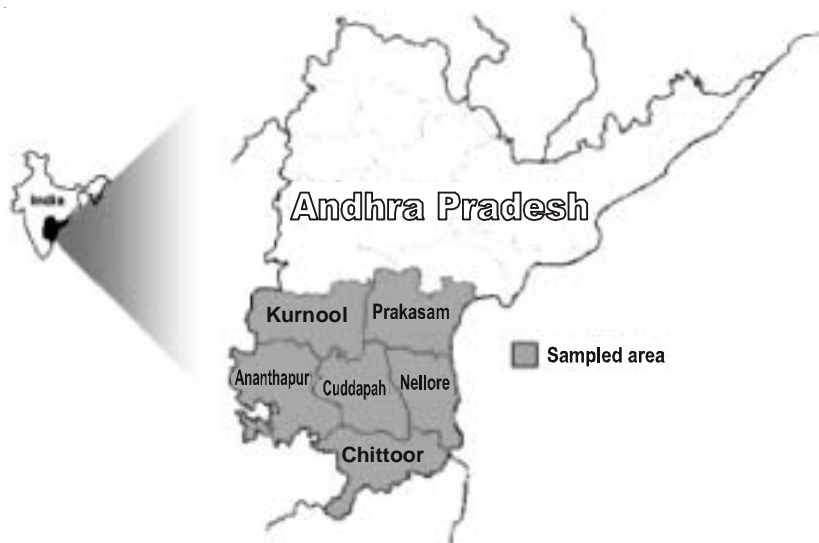
genotypes, particularly the presence of an *ADH2*2* allele, is related to differences in alcohol-drinking behaviour. Among Chinese living in Taiwan, the *ADH2*2* allele was found to be substantially more common in the nonalcoholic group than in the alcoholics (Thomasson et al. 1991). Similar findings have been reported in the Atayal a native population of Taiwan (Thomasson et al. 1995), the Maori of New Zealand (Chambers et al. 2002), Spanish patients (Borras et al. 2000) and among Jews living in the USA or in Israel (Neumark et al. 1998). There was no apparent effect of *ADH2* alleles on the quantity and frequency of drinking in Japanese men (Takeshita et al. 1994), although the number of individuals with the genotypes expected to predispose to the highest consumption (individuals homozygous for *ADH2*1*) is small because of the allele frequencies in this population. A more recent study however indicated that *ADH2*1* was more common in heavy drinkers than in moderate drinkers (Tanaka et al. 1997). On the other hand, in Asians, the *ADH3*1* allele was more prevalent in the non-alcoholics than in the alcoholics (Shen et al. 1997), but there is no apparent effect of the *ADH3* locus on alcohol consumption or alcoholism rates in Caucasians (Gilder et al. 1993). However, the mechanism behind the protective effect of *ADH3* gene is uncertain. Since the *ADH2*2* allele encodes the highly active $\beta 2$ *ADH* isozyme, it has been postulated that faster conversion of alcohol to acetaldehyde could be 'protective' against heavy drinking and alcoholism, although alcohol elimination rates and peak blood acetaldehyde levels were not influenced by the *ADH2*2* genotype (Mizoi et al. 1994). Fewer studies on the relationship between the *ADH2*3* allele and risk of alcoholism or drinking behaviour have been carried out. The presence of *ADH2*3* was associated with a negative family history of alcoholism (Ehlers et al. 2001) and with greater alcohol expectancies (Ehlers et al. 2003). However, the *ADH2*3* allele was not found at a different frequency in alcoholics and controls in a study of more than 200 African-Americans (Taylor et al. 2003). The *ADH3*1* allele is most prevalent among East Asians and Africans (about 90%) and is about equally distributed with *ADH3*2* in Caucasians (Agarwal and Goedde 1992). A critical evaluation of the earlier studies on the nature and extent of the association of the above polymorphisms with alcoholism, are far less

conclusive for Caucasians because of the very low frequency of *ADH2*2* allele (Vidal et al. 2004). In addition, there is evidence that *ADH2*2* and *ADH3*1* alleles are in linkage disequilibrium (Chen et al. 1999; Osier et al. 1999), which clearly indicates that their contribution to the associated risk of alcoholism is not independent.

Pertinent to the Indian subcontinent, while Goedde et al. (1992) reported 9.9% *ADH2*2* allele in a heterogeneous sample of Indians, Osier et al. (2002) reported 6.6% of *ADH2*2* allele in the Kachari population of Assam. These studies therefore cannot reflect the predominant pattern of distribution of *ADH* genes in the Indian subcontinent, which is contiguous to China and certain other Asian countries where some of these alleles show not only strong association with alcoholism but also high frequency of certain *ADH* alleles. In this paper, we report single nucleotide polymorphism at the 4 sites, three from the *ADH2* (1B) and one from *ADH3* (1C) genes among the 28 populations from southern parts of India. The studied populations represent almost entire gamut of socioeconomic and occupational variation inherent in the Indian population structure and are clearly stratified into upper, middle and lower castes and tribes. Since the traditional cultural and behavioral patterns of these populations pertaining to habitual drinking etc are known specific to each of them, we further endeavor to examine if the frequency of any of these alleles implicated in alcoholism follow a trend consistent with the known lifestyle patterns among them.

MATERIALS AND METHODS

A total of 1048 individuals belonging to 28 endogamous populations distributed in the contiguous areas of the 6 southernmost districts of Andhra Pradesh (Chittoor, Cuddapah, Ananthapur, Kurnool, Prakasam, and Nellore) (Fig.1) were included in the present study. All participants provided written informed consent and Review Committee of ISI, Kolkata, approved this study. Intravenous blood samples (about 5 ml each) were collected from them. The samples were drawn mostly from high school and college students, who represent a large number of surrounding villages and populations, and were supplemented with samples collected from the villages, particularly for tribes and lower castes. The names of the populations studied, the number



Populations: 1. Brahmin, 2. Kshatriya, 3. Vysya, 4. Akuthota, 5. Kamma, 6. Kapu, 7. Pokanati, 8. Panta, 9. Vanne, 10. Balija, 11. Ekila, 12. Kurava, 13. Thogata, 14. Yadava, 15. Ediga, 16. Gandla, 17. Jangam, 18. Devangapattur, 19. Chakali, 20. Mangali, 21. Vadde, 22. Madiga, 23. Mala, 24. Erukala, 25. Sugali, 26. Yanadi, 27. Dudekula, 28. Sheik

Fig. 1. Map of Andhra Pradesh showing sampled area and the name of populations studied.

of samples drawn from each population, and their socioeconomic backgrounds are furnished in Table 2. Although this study area can be considered culturally, linguistically, and geographically homogeneous, it is inhabited by a wide array of caste and tribal populations, representing almost the entire spectrum of socioeconomic variation in the state. DNA of the above samples was isolated from the peripheral lymphocytes by proteinase K (Sigma, USA) digestion followed by standard protocols with phenol-chloroform extraction and ethanol precipitation.

Genotyping of exon 3 and 9 of *ADH2* and exon 8 of *ADH3* (Table 1) involved PCR and sequencing. The PCR conditions and primers were as described earlier (Osier et al. 2002). PCR was carried out in a GeneAmp 9600 Thermal cycler (Perkin Elmer) in 10 μ l volume and using 1.0 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR amplicons

were checked on 2% agarose gel. PCR amplicons (70 ng) were directly sequenced using the BigDye Terminator Cycle Sequencing Kit[®] (Applied Biosystems, Foster City, CA) using 5PM primer (Thangaraj et al. 2003). Extended products were purified by alcohol precipitation followed by washing with 70% alcohol. Purified products were then dissolved in 10 μ l of 50% Hi-Di formamide and analysed in ABI3730 automated DNA Analyser (Perkin Elmer, USA).

Statistical Analysis: Allele frequencies were computed using the Gene Counting method. Genotype distributions were tested for deviation from the Hardy-Weinberg Equilibrium proportions using the HWSIM program. To test the degree of heterogeneity in the genotype frequencies among the hierarchical groups χ^2 tests were also performed. Haplotype and linkage disequilibrium analysis was performed using Arlequin software (Ver. 2.1).

Table 1: The description of the SNPs studied as part of *ADH3* and *ADH2* genes

<i>SNP location</i>	<i>Relative position in gene</i>	<i>Polymorphic Site</i>	<i>Ancestral allele</i>	<i>db SNP ID</i>
ADH3 Exon 8	Ile349Val	G/A TTTTA	G (Val)	rs698
ADH2 Exon 3	Arg47His	G/A CACAGA	G (Arg)	rs1229984
Exon 9	Arg369Cys	C/T GTACC	C (Arg)	rs2066702
3'UTR	-	A/G AGATCT	A	rs17033

Table 2: Genotype and allele frequency of ADH3 and ADH2 genes within the caste and tribal populations of Andhra Pradesh, India

Population (sample No)	Social status	Ile349Ile (ADH3-Exon5)				rs17033 (ADH2-3'UTR)			
		Genotype		Allele frequency		Genotype		Allele frequency	
		Val349Val	Ile349Val	Ile	Val	A/A	G/G	A	G
Brahmin (18)	Upper	6	4	0.556	0.444	16	2	0.944	0.056
Kshatriya (26)	Upper	4	8	0.692	0.308	25	1	0.962	0.038
Vysya (18)	Upper	2	11	0.583	0.417	15	3	0.917	0.083
Akuthota (28)	Upper-middle	4	10	0.679	0.321	27	1	0.982	0.018
Kamma (43)	Upper-middle	2	16	0.767	0.233	40	3	0.965	0.035
Kapu (27)	Upper-middle	4	10	0.667	0.333	27	1	1	0
Pokanati (39)	Upper-middle	4	23	0.603	0.397	38	1	0.987	0.013
Panta (37)	Upper-middle	6	22	0.541	0.459	37	1	1	0
Vanne (30)	Upper-middle	4	11	0.683	0.317	30	1	1	0
Baliya (31)	Lower-middle1	1	11	0.79	0.21	31	1	1	0
Ekila (30)	Lower-middle1	3	14	0.667	0.333	26	4	0.933	0.067
Kurava (31)	Lower-middle1	3	16	0.645	0.355	30	1	0.984	0.016
Thogata (16)	Lower-middle1	1	7	0.688	0.313	15	1	0.969	0.031
Yadava (30)	Lower-middle1	3	13	0.683	0.317	27	3	0.95	0.05
Ediga (30)	Lower-middle2	5	18	0.533	0.467	28	2	0.967	0.033
Gandla (24)	Lower-middle2	4	12	0.583	0.417	24	2	1	0
Jangam (14)	Lower-middle2	3	8	0.5	0.5	14	1	1	0
Devangapattur (59)	Lower-middle2	12	28	0.559	0.441	57	2	0.983	0.017
Chakali (26)	Lower1	2	13	0.673	0.327	26	1	1	0
Mangali (13)	Lower1	3	5	0.577	0.423	13	1	1	0
Vadde (42)	Lower1	5	22	0.619	0.381	40	2	0.976	0.024
Madiga (64)	Lower1	10	22	0.672	0.328	62	2	0.984	0.016
Mala (49)	Lower1	5	23	0.663	0.337	48	1	0.984	0.016
Erukala (75)	Proto-Australoid tribe	15	36	0.56	0.44	75	1	1	0
Sugali (37)	"Caucasoid" tribe	4	14	0.703	0.297	34	3	0.959	0.041
Yanadi (165)	Proto-Australoid tribe	22	71	0.652	0.348	156	9	0.973	0.027
Dudekula (25)	Muslims	2	11	0.7	0.3	24	1	0.98	0.02
Sheik (21)	Muslims	2	4	0.81	0.19	18	1	0.905	0.095

Arg47His and Arg369Cys sites of ADH2 are monomorphic to Arg (ADH2*1) in these populations

Table 3: Genotype and allele frequency of ADH3 and ADH2 genes in the hierarchical populations of Andhra Pradesh, and the goodness of fit χ^2 value for HW equilibrium.

	Ile349Val					rs17033 (3'UTR)					
	Genotype			Allele frequency		Genotype			Allele frequency		HW χ^2
	A/A (Ile349Ile)	G/G (Val349Val)	A/G (Ile349Val)	A (Val349)	G (Ile349)	A/A	G/G	A/G	A	G	
Upper (62)	27	12	23	0.621	0.379	56	1	5	0.944	0.056	3.66
Upper-middle (192)	88	24	92	0.657	0.343	199	0	5	0.988	0.012	0.031
Lower-middle1 (138)	65	11	62	0.696	0.304	129	0	9	0.967	0.033	0.157
Lower-middle2 (127)	37	24	66	0.551	0.449	123	0	4	0.984	0.016	0.033
Lower1 (194)	84	25	85	0.652	0.348	189	0	5	0.987	0.013	0.033
Proto-Australoid tribe (277)	115	41	121	0.634	0.366	265	0	12	0.978	0.022	0.136
Muslims (46)	27	4	15	0.75	0.25	42	1	3	0.946	0.054	6.146

RESULTS AND DISCUSSION

At the protein level the allelic series for ADH1B (ADH2) is generated by variation at two different sites at the genomic level: ADH2*1 (ADH1B*1) allele is composed of 47Arg and 369Arg, ADH2*2 (ADH1B*2) allele is composed of 47His and 369Arg and ADH2*3 (ADH1B*3) allele is composed of 47Arg and 369Cys. On the other hand, the two alleles for ADH3 (ADH1C) is generated by the substitution of isoleucine at 349 (ADH3*1) to valine (ADH3*2). Out of the 4 sites sequenced (Table 1) two (Arg47His & Arg369Cys) were found to be totally absent in these populations, hence monomorphic for ADH2*1 (composing 47Arg and 369Arg). The ADH2*2 allele which is known to confer protection against alcoholism and found in high frequency in most East Asian populations was not found in any of the 28 populations that we screened for this study. The frequency of this allele (*ADH2*2*) was observed to be > 33% in East Asian populations and less than 25% in other regions (Osier et al. 2002). Given the probable East Asian admixture, the frequency reported for Kacharis of Assam in India (6.6%, *N* = 30) (Osier et al. 2002) may be in order. As expected, the *ADH2*3* (ADH2*369Cys) which is essentially an African specific allele is also absent in the populations of the present study.

At the other two sites, which are polymorphic, none of the 28 populations of this study deviated significantly from Hardy Weinberg Equilibrium proportions after bonferroni correction (Table 2). At the *ADH3*1* Ile349Val site, the high activity allele (*ADH3*1*) is more frequent among these populations, ranging from 0.500 to 0.810. However, ADH2 3'UTR- rs17033 (72 bases down stream of ADH2 Arg369Cys) is polymorphic only in 19 of the 28 populations and the frequency of the derived allele (G) range from 0 to 10%. The degree of heterogeneity in the genotype frequencies among the hierarchical groups (Table 3) is significant for Ile349Val (df = 12; $\chi^2 = 22.050$) and not for the 3'UTR rs17033 (df = 6; $\chi^2 = 9.765$). However, the allele frequencies do not show any clear trend across socioeconomic groups. Out of 4 possible haplotypes constructed using the 4 sites (Fig. 2), AGCA is the major haplotype followed by GGCA, AGCG and GGCG. The distribution of these haplotypes among the hierarchical groups is found to be highly

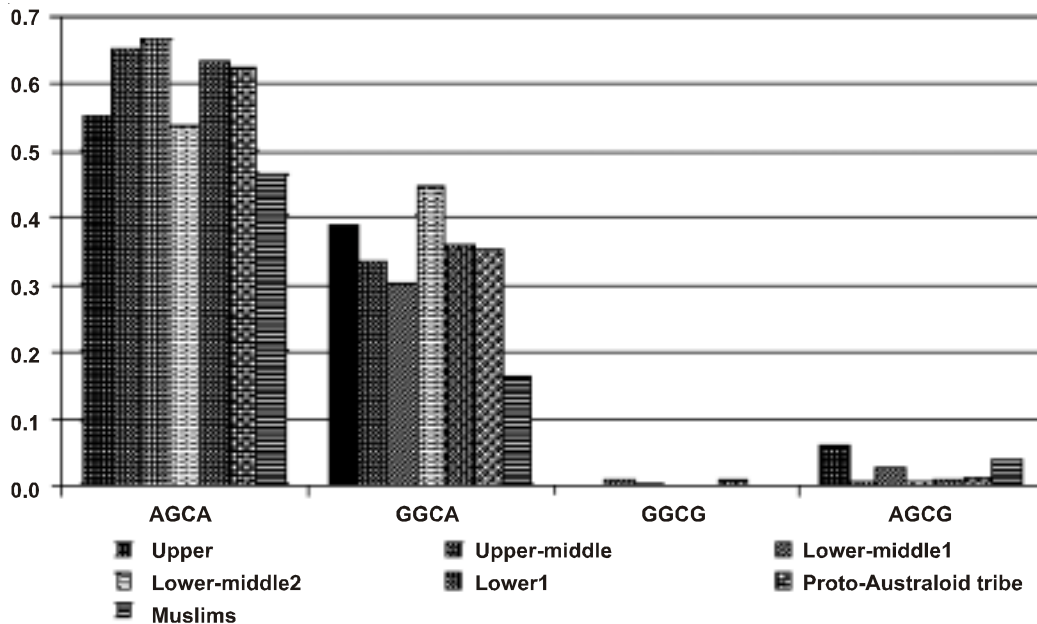


Fig. 2. Histogram depicting relative frequency of haplotypes based on the SNPs at the ADH3 and ADH2 genes. Given that two out of 4 sites are monomorphic, only four haplotypes are possible.

homogeneous and statistically nonsignificant ($\chi^2 = 0.248$, $df = 18$). Linkage disequilibrium does not exist between the two-polymorphic loci.

While the complete absence of African specific ADH2*3 allele in the Indian populations is expected the total absence of ADH2*2 allele in these populations is somewhat intriguing given that it is found in the neighboring East Asian populations with high frequency and known to confer protection against alcoholism. Does this mean that this allele has no selective advantage/role among the populations of India by way of preventing consumption of alcohol? Most traditional Indian populations that are particularly dependent on manual labor/intense physical activity are known to be habitual consumers of alcohol and these behavioral/lifestyle patterns have been well entrenched into the cultural patterns of the Indian hierarchical society so much so that the upper castes have traditional obligation to sponsor the members of service castes/lower castes for their expenses on drinking during certain religious occasions. Further, most of these populations in the lower rung of the Indian social system spend considerable amount of their earnings through hard physical labour

on drinking. Therefore, the Indian populations might have developed genetic adaptation to withstand drinking unlike the most East Asian populations with known sensitivity to alcohol consumption, hence possibly requiring no protective mechanism, hence the ADH2*2 allele. It may be pertinent to recall that Carr et al. (2002) studying ADH2 polymorphisms in 4 groups of Jewish subjects in college age and in general population observed that college students consumed considerably more alcohol than the general population suggesting that social setting and age have a stronger influence on alcohol drinking than the effect of ADH2*2 allele.

Although ADH3*1 allele is observed in high frequency among these southern Indian populations, its association with alcoholism is said to be due to linkage disequilibrium with ADH2*2, rather than due to its direct role on the metabolic activity. Even so, given complete absence of ADH2*2 allele in these populations, hence monomorphic nature of ADH3*1, and in the absence of any pattern in ADH3*1 or ADH3*2 allele frequencies across hierarchical groups with known alcoholic consumption behaviors, no tangible interpretation can be

deduced for the high frequency of this allele and its role in alcoholic behaviors. Nevertheless, our study may partly fill in the lacunae in the knowledge on the nature of distribution of ADH genes among the Indian populations.

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