Association of *ADH1B* and *ALDH2* gene polymorphisms with alcohol dependence: A pilot study from India

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Abstract

Functional polymorphism in the genes encoding alcohol dehydrogenase (ADH) 1B and aldehyde dehydrogenase (ALDH) 2 are considered most important among several genetic determinants of alcohol dependence, a complex disorder. There is no report on the widely studied Arg47His and Glu487Lys polymorphisms from Indian alcohol-dependent populations. In this paper, we report, for the first time, allelic and genotypic frequencies of Arg47His and Glu487Lys single nucleotide polymorphisms (SNPs) in North Indian alcohol-dependent subjects. A total of 174 alcohol-dependent males, recruited using DSM IV criteria (American Psychiatric Association, 1994), were genotyped using the polymerase chain reaction–restriction fragment length polymorphism method. The results obtained from genetic analysis were correlated with clinical parameters using Student's t-test or Mann Whitney's U test. The highlight of the study findings was the uniquely high frequency of the *ALDH2**2/*2 genotype (among alcohol-dependent subjects) being a risk-conferring factor for alcohol dependence.

Keywords: *alcohol dependence, alcohol dehydrogenase, aldehyde dehydrogenase, genotype–phenotype correlation*

Introduction

Alcohol dependence (AD; Mendelian Inheritance in Man, (MIM) %1037800), a chronic relapsing disorder, is a serious health concern globally. It is characterised by loss of sensitivity and development of tolerance to, and withdrawal symptoms and craving for alcohol.[1] In addition, AD also leads to a plethora of disabling complications, such as hepatitis, hepatic cirrhosis, chronic pancreatitis, testicular atrophy and avascular necrosis of the hip joint.[2] The morbidity, mortality and high cost associated with the treatment of alcoholism and its related complications makes AD a major socio-economic burden on society.

Alcohol abuse disorders result from the interaction between an individual's genetic and environmental susceptibility and repeated intake of alcohol over time. It is not possible to become alcoholic without repeatedly consuming alcohol, but only a small percentage of all drinkers become alcoholic.[3] Epidemiological studies suggest that ethnicity and genetic susceptibility are crucial in the genesis of AD,[4–10] with American Indians, Native Hawaiians, African Americans and Hispanics being highly susceptible to developing AD.[11]

Although the prevalence of AD in the Asian population is low, a sizable number of people in India are suffering from alcohol use disorders.[12] Genetic predisposition to AD, conferred by various candidate genes, differs across ethnic groups.[13] This differential contribution leads to inconsistent genetic association and non-replication of association between different populations. Linkage and association studies to determine genetic factors

predisposing to AD have been carried out in several Western, African, Jewish, Korean, Japanese and Chinese populations.[14,15] Although India represents about one-sixth of the world's population, there are very few data on genes/polymorphisms that confer susceptibility to AD in this population.

Genetic polymorphisms, particularly those of the alcohol metabolising enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), have been largely implicated in the development of AD.[16–18] In order to identify specific genes affecting vulnerability or resistance, Long *et al.*[16] performed a whole-autosomal genome scan for genetic linkage to AD in a Southwestern American Indian tribe. A highly suggestive piece of evidence for the linkage of three loci in the *ADH* gene cluster on chromosome 4q was observed. These findings were confirmed by the Collaborative Study on the Genetics of Alcoholism (COGA) study.[17] On the basis of the genotype frequencies of *ADH1B* and *ALDH2* polymorphisms, the risk for alcoholism in the Japanese population was accurately estimated by Higuchi *et al.*[19] They concluded that the Japanese population may be protected from alcoholism by inactive ALDH2 and higher frequencies of atypical ADH1B.

Functional polymorphisms in the genes encoding these enzymes influence the rate of synthesis and metabolism of acetaldehyde, the toxic metabolite of ethanol. The presence/absence of these polymorphisms in the population could modulate the probability of the development and relapse of AD, and confer protection/susceptibility to alcohol abuse and alcohol-related complications. The most widely studied functional polymorphisms in the ethanol metabolic pathway are the *ADH1B* Arg47His and *ALDH2* Glu487Lys polymorphisms.[20]

The *ADH1B*2* (*ADH1B*47His*) allele codes for a higher activity enzyme as compared with the *ADH1B*1* (*ADH1B*47Arg*) allele[21,22] and is thus known to influence drinking behaviour, resulting in protection from alcoholism. Chen *et al.*,[23] in a case control analysis, found that individuals carrying one or two copies of the *ADH1B*2* allele and a single copy of *ALDH2*2* had the lowest risk (odds ratios 0.04–0.05) for alcoholism, as compared with the *ADH1B*1/*1* and *ALDH2*1/*1* genotype. Further, the authors concluded that the disease risk associated with the *ADH1B*2/*2–ALDH2*1/*1* genotype is about half of that associated with the *ADH1B*1/*2–ALDH2*1/*1* genotype. Their result suggests that the protection afforded by the *ADH1B*2* allele may be independent of that afforded by *ALDH2*. A considerable variation in the allele frequency of this polymorphism has been observed among different ethnic groups.[22] The *ADH1B*2* allele is found to be more common in non-alcoholic than in alcoholic groups in populations from East Asia, Taiwan, Spain, New Zealand and in Jews from Israel and the USA. Due to the very low frequency of the *ADH1B*2* allele in the Caucasian population, however, association studies have mostly remained inconclusive.[24] Reports on the *ADH1B*2* allele (Arg47His polymorphism) frequency in the Indian population are inconsistent. Goedde *et al.*[25] reported a 9.9 per cent *ADH1B*2* allele frequency in a heterogeneous sample from the Indian population, whereas another study from India (on the Kachari population) reported a 6.6 per cent frequency.[21] Recently, in a robust study involving 28 Indian tribal populations, the polymorphism has been found to be monomorphic (*ADH1B*1/*1*), with complete absence of the *ADH1B*2* allele.[24] Based on their findings, those authors argued that Indians (mainly those comprising the lower caste and working class) could have developed tolerance to the adverse effects of alcohol, and thus the protection-conferring *ADH1B*2* allele has been selected out from the population.

Mitochondrial ALDH2 plays a major role in ethanol metabolism. It is involved in the oxidation of acetaldehyde to acetate. Sensitivity to ethanol is highly associated with a functional polymorphism, Glu487Lys, and the 487Lys (*ALDH2*2*) allele is responsible for a deficiency in ALDH2 activity.[26] High concentrations of acetaldehyde, due to the presence of the *ALDH2*2* allele, lead to adverse reactions to alcohol,[27] which reduce the probability of heavy drinking *vis-à-vis* AD and other alcohol-related problems. Clinical and pharmacokinetic studies have indicated that individuals who show

initial sensitivity to alcohol by virtue of their genetic make-up (ie the presence of the *ALDH2*2* allele) are discouraged from drinking excessive amounts of alcohol.[28] Assanangkornchai *et al.*[29] found that *ALDH2*2/*2* subjects are at minimum risk for developing AD and alcohol-related organ damage, compared with those with the *ALDH2*1/*2* and *ALDH2*1/*1* genotypes.

A significant variation in the allele frequency of the Glu487Lys polymorphism has been observed across different populations worldwide, with almost complete absence of the *ALDH2*2* allele in Caucasians, Africans and Americans (single nucleotide polymorphisms [SNP] database; National Center for Biotechnology Information) and a relatively higher frequency in East Asians. Studies on Chinese and Korean populations found that alcoholics are less likely to have the *ALDH2*2* allele than controls.[23,30,31] Pertinent to the East Asian population, approximately 2–12 per cent of Chinese and Korean individuals are homozygous for the *ALDH2*2* allele (*ALDH2*2/*2* genotype) and appear to be almost completely protected.[23] The Glu487Lys polymorphism, however, is found to be monomorphic in Indian populations from Madhya Pradesh, Maharashtra and Andhra Pradesh.[28]

Due to the paucity of Indian data with regard to *ADH1B/ALDH2* gene polymorphisms, the present study aimed to characterise the Arg47His and Glu487Lys polymorphisms in Indian subjects with AD and to establish the genotype/phenotype correlation (if any).

Materials and methods

Subjects

In this study, 174 alcohol-dependent male subjects satisfying DSM 1V criteria derived from the Diagnostic Interview for Genetic Study (DIGS), and aged between 18 and 60 years of age, from the National Drug Dependence Treatment Center at the All India Institute of Medical Sciences, were recruited. Subjects with a history of any other substance abuse/dependence were excluded. The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Ethical committee clearance was obtained prior to recruitment of subjects. All subjects gave written informed consent to participate in the study. Clinical details, including ethnicity, family history, age at first use of alcohol, quantity of alcohol consumed (g/day), duration of alcohol use, duration of AD, age at onset of dependence, presence/absence of delirium and any other psychiatric or physical illness were assessed and recorded. Serum proteins (albumin, bilirubin, glutamic oxaloacetic transaminase [SGOT] and glutamic pyruvic transaminase [SGPT]) were estimated on an autoanalyser, using biochemical kits from Boehringer Mannheim (Mannheim, Germany).

Genomic DNA extraction and genotyping

Peripheral blood (6 ml) was collected in sterile ethylene diamine tetra-acetic acid (EDTA)-coated vacutainers and stored at 4°C until processing for DNA extraction was carried out. Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol–chloroform method. SNP genotyping was done using the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) approach. Forward and reverse primer sequences used for the amplification of the *ADH1B* Arg47His SNP were 5′-AATCTTTTCTGAA TCTGAACAG-3′ and 5′-GAAGGGGGGTCACCA GGTTGC-3′, respectively; and primer pairs for *ALDH2* Glu487Lys were 5′-CAAATTACAGGGT CAAGGGCT-3′ (forward) and 5′-CCACACTCA CAGTTTTCTCTT-3′ (reverse). After amplification, the PCR product (20 μl) was divided into two equal parts. 10 μl of the amplified product was incubated at 37°C for two hours with NmuCI and Mbo II restriction enzymes (RE) for *ADH1B* and *ALDH2*, respectively. DNA fragments in the RE digested and undigested mixture were analysed on 10 per cent polyacrylamide gel electrophoresis (PAGE) using one per cent tris-borate-EDTA (TBE) buffer and sized with the help of known DNA markers. For the *ADH1B* polymorphism, the presence of 95 base pair (bp) and 65 bp restriction fragments indicated the presence of wild-type Arg and mutant His alleles, respectively. The presence of 134 bp and 123 bp

fragments indicated the presence of wild-type Glu and mutant Lys alleles, respectively for the *ALDH2* SNP. Genotyping for approximately 10 per cent of the samples was repeated at random for quality control, and complete agreement was found.

Statistical analysis

Comparison of all clinical variables between subjects with different genotypic profiles for *ADH1B* and *ALDH2* gene polymorphisms were carried out using the t-test or Mann Whitney's U test, as appropriate; p values <0.05 were considered significant.

Results

The clinical details of the study population are presented in Table 1. The population was divided into three groups based on patients' genotypic profile for *ALDH2* (Glu487Lys) gene polymorphism, and the distribution of clinical variables was evaluated.

ADH1B gene polymorphism was found to be largely monomorphic, with a minor allele frequency (*ADH1B*2*) of 0.0014.

For the *ALDH2* Glu487Lys SNP, the genotypic frequencies were 0.73 (*2*1/*1*), 0.16 (*2*1/*2*) and 0.11 (*2*2/*2*), with a minor allele frequency (*ALDH2*2*) of 0.19. The significance of association of various clinical parameters with *ALDH2* polymorphism is included in Table 1.

About 80 per cent of subjects with the *ALDH 2*1/*2* genotype, and 38 per cent with the *ALDH2*1/*1* genotype reported a history of severe alcohol withdrawal (delirium) during their alcohol drinking years. About 39 per cent of those

Table 1. Clinical details of the study population

Population characteristic	Mean ± SD (n = 174)	*ALDH2* (Glu487Lys) genotype			p
		*1/*1 (n = 128)	*1/*2 (n = 27)	*2/*2 (n = 19)	*1/*1 *vs.* *2/*2
Quantity of alcohol consumed (g/day)		200 – 400	130 – 200	30 – 50	
Age at first use of alcohol (years)	21.2 ± 5.89	20.27 ± 6.54	20.14 ± 6.12	23.87 ± 3.833	0.067
Duration of alcohol use (years)	14.8 ± 9.36	17.00 ± 12.11	12.42 ± 3.69	12.75 ± 6.11	0.194
Duration of dependence (years)	7.53 ± 7.19	10.33 ± 8.44	6.07 ± 5.23	3.56 ± 3.31	<0.01[a]
Age at onset of dependence (years)	25.9 ± 8.17	22.33 ± 4.70	26.5 ± 8.27	32.06 ± 10.12	<0.01[a]
Duration of alcohol use prior to development of dependence (years)	7.3 ± 5.99	6.67 ± 6.99	6.36 ± 3.17	9.19 ± 6.09	0.33
SGOT (IU)	58.38 ± 63.92	66.63 ± 75.51	56.93 ± 53.38	36.12 ± 16.72	0.011[a]
SGPT (IU)	47.23 ± 53.09	54.04 ± 65.60	42.18 ± 30.24	32.68 ± 20.95	0.041[a]
Total protein (g%)	0.62 ± 0.16	0.59 ± 0.16	0.64 ± 0.08	0.64 ± 0.22	0.45
Albumin (g%)	6.9 ± 0.71	6.88 ± 0.73	6.84 ± 0.84	7.08 ± 0.51	0.20
Bilirubin total (mg%)	4.65 ± 5.33	4.09 ± 0.49	6.88 ± 11.78	4.04 ± 0.44	0.69

[a]Significant at $p < 0.05$.
SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase

with the *ALDH2*1/2*1* and 29 per cent of those with the *ALDH2*1/*2* genotype drank almost every day. Subjects drinking excessive amounts (>60 g/day), however, were more likely to have the *ALDH2*1/*1* or the *ALDH2*1/*2* genotype than the ALDH2*2/*2 genotype.

Discussion and conclusion

The pharmacokinetics of ethanol metabolism influences the risk for AD. Although the role of *ADH1B* and *ALDH2* genes in susceptibility to alcoholism were discovered individually, they have been shown to act additively when they co-occur. The *ADH1B*2* and *ALDH2*2* alleles raise the levels of acetaldehyde by increasing the rate of synthesis and decreasing the rate of metabolism respectively, thus interacting additively but not synergistically.

At the protein level, the allelic series for ADH1B is generated by variation at two different sites at the genomic level. The *ADH1B*1* allele is composed of 47Arg and 369Arg; the *ADH1B*2* allele is composed of 47His and 369Arg; and the *ADH1B*3* allele is composed of 47Arg and 369Cys. Caucasians are largely monomorphic for the *ADH1B*1* allele,[25] with a very low frequency of the *ALDH2*2* allele. The *ADH1B*2* allele, which is known to confer protection against alcoholism and is relatively common in East Asian populations,[21] was found to be present at very low frequency (<0.001) in our study. Our observation is similar to that reported by Reddy *et al.*[24] in tribal populations from southern India. Since the *ADH1B*3* (*ADH1B*369Cys*) allele has been documented to be essentially specific to the African population, we did not genotype *ADH1B*3* in our study population.[24]

Although consistent with the recent report by Reddy *et al.*,[24] the very low frequency of the *ADH1B*2* allele found in our population is surprising, since it is present at a substantial frequency in the neighbouring East Asian population and confers protection against alcoholism. A very low frequency of *ADH1B*2* could indicate a selection pressure operating against the *ADH1B*2* allele in our population due to its inability/redundancy to protect the Indian population from AD. Commenting on the alcohol-consuming practice of the service/working and lower caste of Indian society, Reddy *et al.*[24] suggested that, due to the heavy amount of alcohol consumption on a regular basis, most of these subjects might have developed a genetic adaptation to withstand drinking alcohol (unlike most East Asian populations with known sensitivity to alcohol consumption), thereby possibly requiring no protective mechanism and hence resulting in the absence of the *ADH1B*2* allele. The present study was conducted at a government facility, where treatment is available free of charge and is accessed mainly by people from low income strata belonging to the working class of Indian society. It is well known that socio-economically underprivileged groups consume alcohol for mental and physical relaxation. Therefore, the explanation given by Reddy *et al.*[24] could also support a very low frequency of the *ADH1B*2* allele in the present study. A stronger influence of social setting and age than the effect of the *ADH1B*2* allele on alcohol drinking has also been found in a study in a Jewish population,[32] which supports the above-mentioned hypothesis of an environmental influence on genetic constitution with regard to the *ADH1B* gene in the Indian population.

The *ALDH2* gene is located on chromosome 12q24. The single bp difference (G > A; Glu487Lys) in exon 12 causes the normal allele, *ALDH2*1*, to become a non-functional (*ALDH2*2*) allele, which codes for the inactive enzyme.[6] The *ALDH2*2* allele, which is prevalent in Asian populations but is extremely rare in non-Asians, has the strongest protective association with AD. Asian subjects homozygous for *ALDH2*2* have an almost zero risk of developing AD, whereas heterozygotes (*ALDH2*1/2*2*) are about one-third as likely to be alcoholic, compared with those without this allele.[14] Assanangkornchai *et al.*[29] reported that the homozygous *ALDH2*2/*2* genotype is present at a very low frequency (0–0.3 per cent) in alcoholic patients, compared with 3–12 per cent among non-alcoholic subjects. The heterozygous *ALDH2*1/*2*

genotype has been found to be present in 6–17 per cent of alcohol-dependent subjects, compared with 30–45 per cent of non-alcoholic subjects.[5,33,34] This difference in *ALDH2*2* allele frequency between alcoholic and non-alcoholic subjects has been postulated to be responsible for the phenomenon of adverse responses to alcohol consumption, such as facial flushing, nausea and tachycardia in individuals with the inactive form of the enzyme.[29] In contrast to the reports from the neighbouring East Asian population, the present study found comparatively higher allele ($ALDH2^*2 = 0.19$) and genotypic ($ALDH2^*2/^*2 = 0.11$, $ALDH2^*1/^*2 = 0.16$) frequencies in alcohol-dependent individuals. A recent study[28] from India is equally exciting, however, as it found the SNP to be monomorphic (*ALDH2*1/2*1*) in healthy control subjects. In light of the findings of that recent report,[28] as well as that of Goedde *et al.*,[35] the higher frequency of the inactive *ALDH2* allele observed in our Indian alcohol-dependent subjects, compared with the control population, provides evidence for a direct role of acetaldehyde in the development of alcohol addiction (despite its toxic actions). Further, the amount of alcohol consumed by *ALDH2*2/*2* individuals is significantly lower than those with *ALDH2*1/*1* and *ALDH2*1/*2* genotypes (Table 1). It should be noted that, although subjects with the *ALDH2*2/*2* genotype in this study are classified as alcohol dependent by DSM IV criteria, these individuals are only drinking three to five restaurant glasses per day (of Indian liquor, with 8 g alcohol/drink), which is not enough to produce ethanol intoxication, but rather acetaldehyde intoxication. When we compared our findings in alcohol-dependent subjects with those of an Indian control population (see Bhaskar *et al.*[28]), our findings were in concurrence with those of Hahn *et al.*,[36] where the pleasurable effects of acetaldehyde led to higher positive expectancies in individuals with the *ALDH2*1/*2* compared with the *ALDH2*1/*1* genotype. As suggested by Chen *et al.*,[37] the pleasurable effects of acetaldehyde leading to higher positive expectancies in individuals with the *ALDH2*1/*2* compared with the *ALDH2*1/*1* genotype could be due to a development of tolerance to acetaldehyde. The present observation of the development of AD (in *ALDH2*2/*2* individuals) with low levels of alcohol consumption but with presumably high levels of acetaldehyde strongly implicates acetaldehyde in the causality of the dependence. The above conclusion might be weakened/challenged, however, by a comparison of our data on AD subjects with an analysis involving ethnicity, age and sex-matched controls; this is currently ongoing in our laboratory.

Two clinical parameters — namely, duration of AD and age at onset of AD — correlated well with the protection-conferring property of the *ALDH2* Glu487Lys polymorphism. The duration of AD was found to be significantly longer ($p < 0.01$) among individuals with the *ALDH2*1/*1* genotype, compared with *ALDH2*2/*2* subjects. The age at which patients developed AD, however, was significantly lower ($p < 0.01$) in individuals with the *ALDH2*1/*1* genotype compared with *ALDH2*2/*2* subjects (Table 1). Alcohol-induced flushing is inherited as a dominant trait in Asians.[38] A substantial number of Asians (50–80 per cent), compared with Caucasians (3–12 per cent), exhibit flushing.[39,40] Acetaldehyde creates unpleasant adverse reactions by acting as a major deterrent to excessive alcohol drinking on the one hand, while giving the euphoric sensations that may reinforce alcohol drinking on the other.[41]

Since people with the inactive *ALDH2*2* allele drink less often and consume less alcohol per occasion than those with the active *ALDH2*1* allele, our observation of a significantly lower age at onset and longer duration of AD among individuals with the *ALDH2*1/*1* genotype compared with those with the *ALDH2*2/*2* genotype seems to be in keeping with the available literature. Significantly higher values of SGOT and SGPT in individuals with the *ALDH2*1/*1* genotype, compared with the *ALDH2*2/*2* genotype (Table 1), is suggestive of more liver damage due to higher alcohol consumption among *ALDH2*1/*1* subjects.

Our findings indicate that functional polymorphism of the gene coding for the ALDH enzyme affects the propensity to develop AD. The most important finding of the study was the

uniquely high frequency of the *ALDH2*2/*2* genotype (among alcohol-dependent subjects) being a risk-conferring factor for AD. Further, based on the findings from this study, albeit based on a limited sample size, it could be hypothesised that the Indian genotype is prone to slow metabolism of alcohol and high metabolism of acetaldehyde, and thereby prone to alcoholism. Our findings should be viewed, however, in the perspective of the potential limitation posed by the absence of data from an ethnically, age- and sex-matched control population. Further, these results not only warrant replication in larger sample sets, but also underscore the need for investigations on candidate gene polymorphisms from other biochemical pathways.

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