Association between ALDH2 Variant and Alcohol Dependence in South Indian Population.

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Abstract

Background: Alcohol dependence (AD) poses a serious medical problem and significant public health issue contributing to morbidity and mortality throughout the world. The aim of the study is to test the association of rs698 (*ADH1C*) and rs671 *(ALDH2*) with the risk of alcohol dependence and to further assess the influence of environmental factors on altering the genetic susceptibility to alcoholism in south Indian Tamilian population.

Methods& Results: A total of 150 alcohol dependent cases aged between 18- and 65-years fulfilling DSM-V criteria were recruited from de addiction center. Subjects in control group (n=150) had history of alcohol intake with AUDIT score less than 8. The alleles were genotyped using TaqMan SNP genotyping assays by quantitative PCR. Association with alcohol dependence was evaluated with various genetic models using chi-square test. Multiple logistic regression analysis was performed to explore the effect of covariates. The dominant (OR=0.5811,95% CI: 0.372-0.9224, p<0.01) and allelic genetic model (OR=0.6228,95% CI: 0.4328-0.9009, p<0.01) of *ALDH2*, rs671 between cases and controls showed a significant association of the genetic variant with AD. Multivariate logistic regression analysis revealed education level, family history, marital status were significantly associated with AD but there was no association between rs671 genotypes in the presence of these co-variates.

Conclusions: Genetic factors play an important role in alcohol dependence. Combined analysis of functional genetic variants and environmental factors is warranted in future studies.

Introduction

Alcohol dependence (AD) is a multifactorial chronic relapsing behavioral disorder and a major public health problem [1]. Heterogeneity in alcoholism is influenced by genetic factors and further moderated by environmental and social factor [2]. Findings from many families, adoption, and twin studies on alcoholism revealed that genetic factors [3] play a considerable role in the development of alcohol dependence where risk proportion ranges from 40 to 60% in both males and females[4]. Alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) are the primary enzymes responsible for the metabolism of ethanol. The *ADH* and *ALDH* isoforms that determine the levels of acetaldehyde in the body are mainly implicated in the etiology of alcoholism and drinking behavior[5,6]. The aversive effects of acetaldehyde such as facial flushing, nausea and tachycardia reduce the dependence of alcohol and protect against alcoholism [6–8]. A more active *ADH* and a less active *ALDH* may lead to higher accumulation of acetaldehyde in the blood resulting in “flushing syndrome”. Genetic variants in these genes are modifiers of risk of alcoholism [9,10].

The *ADH1C* belongs to class I of human *ADH* genes cluster on chromosome 4q21 [11] and is responsible for about 70% of total ethanol oxidation [6,12].The largest genome-wide association study (GWAS) identified the *ADH1B/ADH1C* region to be associated with the risk of alcoholism [13]. *ADH1C*2*associated variants rs698 (Ile350Val) and rs1693482 (Arg272Gln) which are in very high linkage disequilibrium with

each other [14] have been shown to modify the risk of alcohol dependence mostly in East Asians [15,16]. *ADH1C*1*Ile350 'T' allele which is widely prevalent (about 90%) in Asians has higher enzyme activity about twice the enzyme activity of *ADH1C*2*, and is found to be protective initially in Chinese and Koreans [15–17]. Later compelling evidence was produced by studies across European, American and African populations demonstrating the association between rs698 and alcohol dependence or abuse further corroborated by a meta-analysis [18].

The *ALDH2* gene located on chromosome 12q24.2 encodes the mitochondrial isozyme *ALDH2*, highly expressed in stomach and liver and is significantly involved in the oxidation of acetaldehyde [19].The functional polymorphism rs671, G>A, Glu504Lys also referred to as *ALDH2*2* exhibits lower enzyme activity. The mutant protective allele *ALDH2*2* Lys504 is commonly distributed among Asians ranging in frequency between 12 to 41% [6,20]. Individuals homozygous for this variant have no enzyme activity and heterozygous individuals show 30 to 50% activity [21]. Previous reports have consistently shown the protective effect of *ALDH2*2* in east Asians [22–24].

Genetic variants of *ADH1C* and *ALDH2* were demonstrated to be significantly associated with AD especially in East Asian population including a meta-analysis[15,24–26]. The association between the well-documented variants rs698 and rs671 is much less explored especially in south Indian population. The aim of the present study is to test for association of the two rs698 and rs671 with the risk of alcohol dependence in south Indian Tamilian population as well as study the influence of environmental factors such as education level, employment and marital status on altering the genetic susceptibility to alcoholism.

Methods

Subjects

One hundred and fifty male subjects satisfying Diagnostics and Statistical Manual (DSM)-V criteria for alcohol dependence, aged between 18 and 65 years were recruited from de addiction centre (TTK Hospital, Chennai). Subjects with substance abuse disorder (other than nicotine) and psychiatric illnesses such as schizophrenia, bipolar disorder and, major depressive disorder were excluded. The controls consisted of 150 male subjects with exposure to alcohol but no alcohol use disorder/abuse as evaluated by DSM V as well as Alcohol Use Disorders Identification Test (AUDIT) [27], a useful questionnaire for the identification of harmful drinking where controls are defined by an AUDIT score of less than 8. All subjects were of self-reported Tamilian ancestry.

The study was approved by the Institutional Ethics Committee, Tagore Medical College and Hospital (TMCH), and all the subjects provided written informed consent. Two ml of venous blood was collected from the subjects. DNA was isolated using the spin column-based DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Genotyping

Quantitative PCR was performed using 5' exonuclease fluorescence TaqMan SNP genotyping assay kits (Applied Biosystems, Foster City, CA, USA) for rs698 (assay ID: C_26457410_10) and rs671(C_11703892_10). The real-time polymerase chain reaction protocol comprised of initial denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 15s, annealing & extension at 60 °C for 90s. The assays were run on Bio-Rad CFX96 Real-Time PCR Detection System (Biorad Laboratories, CA, USA). Allelic discrimination was carried out using CFX Maestro Software 2.0 (Biorad Laboratories, CA, USA). TaqMan genotyping was performed using 50ng of DNA, 5 µL of the GoTaq® Probe qPCR Master Mix 2X (Promega Corporation, USA), 0.5 µL TaqMan assay 40X, and 2.5µL of Nuclease-free water. The accuracy of genotyping was confirmed by randomly rerunning 15% of the samples.

Statistical analysis

Demographic variables were compared between cases and controls using chi-square test for nominal variables and student's t-test for continuous variables. Fisher's exact test was used to test if the observed genotype frequencies were in Hardy-Weinberg equilibrium. Allele and genotype frequencies were calculated by direct gene counting method. Association with alcohol dependence was evaluated with dominant, co-dominant recessive and additive genetic models using chi-square test. Binary logistic regression was used to explore the combined effect of rs671 and rs698 alleles on AD. For this analysis, the total number of risk (rs671 'G', rs698 'C') alleles and protective (rs671 'A', rs698 'T') alleles was calculated for each individual. Each subject therefore had either 4 risk alleles or 4 protective alleles or 2 risk and 2 protective alleles or 3 risk alleles and 1 protective allele and vice versa. Multiple logistic regression was performed considering significantly associated variant from univariate analysis and co-variates such as age, family history, education level, employment and marital status.

Results

The demographic characteristics compared between cases and controls are shown in Table 1. We found significantly more educated subjects among controls while number of individuals who had a family history of AD, married and employed were significantly high among alcohol dependents. The study also shows a significantly greater number of smokers among alcohol dependents. Table 2 shows the genotype and allele frequencies of the SNPs. The genotype frequencies of the SNPs were in Hardy-Weinberg Equilibrium (p=0.6242 for rs698; p=0.9327 for rs671). The dominant model of *ALDH2*, rs671 between cases and controls revealed a significant association of the genetic variant with AD. Similarly, the allelic association for rs671 also showed a significant association with AD (Table 3). However, the combined effect of risk and protective alleles did not show any significant association with AD. Multivariate logistic regression analysis showed that factors such as education level (adjusted OR, 95% CI: 6.34, 2.15-18.67), family history (adjusted OR, 95% CI: 2.97, 1.66-5.31), marital status (adjusted OR,

95% CI: 2.75, 1.3-5.78) remained significantly associated with AD but there was no association between rs671 genotypes in the presence of these co-variates.

Discussion

The present study shows the occurrence of the minor alleles of rs671 and rs698 at a frequency between 21-30% and 33-38% respectively in the south Indian study population. The frequency of rs671 across Asian populations (19% Japanese, 23 % Chinese, 36% Koreans) is quite similar to the frequencies observed in this south Indian population. Among Indian populations, intriguingly a study reported Glu504 to be monomorphic in some Indian populations (Madhya Pradesh, Maharashtra and Andhra Pradesh) [28] in healthy controls. However, Vaswani et al. found the *ALDH2*2* minor allele to be present at a frequency of 19% in a north Indian population which agrees with the finding of the present study [29].The minor allele frequency of rs698 in this study population is marginally higher than north Indians (25%) and south West Indians (27%) [14,30]. Across the major global populations, the frequency of this allele is seen at 50% in Caucasians and 15% in African Americans [14] and is the least frequent in east Asians [16,31].

The present study found a significant rs671 allelic association with AD which was also reflected in the dominant genetic model. The strong protective effect of *ALDH2*2* against AD is well-established. A meta-analysis showed a very strong association of rs671 with alcohol dependence and alcohol abuse with an allelic p value of $3x10^{-56}$. The study also showed significance with dominant model (lys-lys + lys-glu vs. glu-glu) concurring with the findings of the present study[6]. The significant association between the decreased risk of alcohol dependence and *ALDH2*2* has been shown in many Asian studies [23,26] including a meta-analysis that demonstrated the link between *ALDH2*1* and increased risk of AD according to a dominant model only in east Asians. O'Shea et al. analyzed the relationship between *ALDH2*2* A allele and peer drinking in east Asian college students confirming the strong protective effect of *ALDH2*2* against alcohol addiction [32]. In an Indian study though, in contrast to studies on Asian subjects, Vaswani et al. found a high frequency of *ALDH2*2* among north Indian alcohol dependent subjects and deemed it to be a risk factor. However, the amount of alcohol consumption in rs671 homozygous mutants was significantly lower compared to other genotypes [29].

More recently, a GWAS (including a discovery and replication cohort) in Han Chinese alcoholic dependents and controls and in their analysis of polygenic risk scores (PRS) derived from GWAS summary statistics with European American, African American and Thai population revealed the significant association of *ALDH2* rs671 and *ADH1B* rs1229984 with AD [33]. In a family-based association test in Taiwanese subjects followed by case-control study and a meta-analysis of both studies, rs671, rs698 and rs1229984 were significantly associated with AD among 282 SNPs and 61

genes involved in the systems of dopamine, serotonin, GABA, and alcohol metabolism [34]. Our study did not find any association between rs698 and AD. The combination of risk and protective alleles from rs671 and rs698 also showed no association with AD.

In an earlier Indian study that reported a considerable frequency of rs698 (about 35%) in north Indian population, no association was found between rs698 and alcoholism [35]. Borras et al., also reported no association between AD and *ADH1C*1* in European individuals [8]. These reports are contradictory to many studies across major global populations where *ADH1C*1*, *Ile350* has been shown to lower the risk of AD [18]. The protective effect Ile350 is consistently reported in different Asian populations [18,36] than in non-Asian populations [24,37–39] However, in a Turkish study, rs698 was not associated with AD whereas rs1229984 (Arg47His, also referred to as *ADH1B*2*) which is in high linkage disequilibrium with rs698 was associated with AD [40]. rs1229984 is also highly prevalent in Asian population but is rarely seen in non-Asians [6,15,41]. rs1229984 is linked with the pathogenesis of alcohol dependence [42] and is protective against AD in different populations [22,42–44].This functional variant encodes β2β2, a more active form of the enzyme that results in rapid oxidation of ethanol and higher concentrations of acetaldehyde [45,46]. It has been suggested that the protective effect of *ADH1C*1* could be in part attributed to its strong LD with *ADH1B*2* [6]. rs698 and the second allelic variant of *ADH1C*2* rs1693482 were also found to be in high LD with rs1789891 which is significantly associated with AD and is located between *ADH1B* and *ADH1C* [47]. There was significant association between *ADH1B* rs129984 and *ADH1C* rs698, rs1693482 variants as well as *ADH1C-1B* intergenic markers and alcohol dependence syndrome in British and Irish population [48].

The variants of the *ADH* cluster are in strong LD with each other to an extent that precludes the analysis of independent effect of these variants [48]. Since the *ADH1* genes are in close proximity with each other, different variants in each of these genes could influence alcoholic behavior. The analysis of joint effect of *ADH1C* and *ADH1B* variants is warranted in future studies [49].

In a study that analyzed the effect of *ADH* variants alongside religious involvement, rs698 was associated with higher maximum drinks and more alcohol dependence symptoms with low or no religious involvement but not with higher religious involvement levels [50], suggesting the significant influence of social factors in modifying the genetic susceptibility. The results of the present study also show the significant influence of social and environmental factors such as family history of AD and level of education in modulating the risk of AD while diminishing the significance of genetic factors in influencing the susceptibility to AD.

Conclusion

The study has corroborated the genetic contribution of *ALDH2*2* conferring protection against AD. However, AD is influenced by many gene-gene and gene environmental interactions. Analysis of joint

effect of different functional genetic variants in conjunction with environmental factors will enable dissecting this complex behavioral disorder for better management and treatment.

Declarations

Competing interests:

Authors state no conflict of interest.

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Ethical approval and Informed consent:

The study protocol was approved by Institutional Ethics Committee of Tagore Medical College and Hospital. Written informed consent was obtained from the participants prior to commencement of the study.

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Tables

Table 1

Demographic characteristics of alcohol dependent cases and controls

	Cases N=150, n(%)	Controls N=150, n(%)	OR (95% CI)	P value
Age in years [a](mean±SD)	37.69±0.828	33.95±1.096		0.0068
Education[b]				
Lower (up to high school)	96(64.0)	52(34.7)	2.251(1.406-3.604)	0.001
Higher	54(36.0)	98(65.3)		
Employment[b]				
Yes	139(92.7)	117(78.0)	3.564(1.720-7.464)	0.0005
No	11(07.3)	33(22.0)		
Cigarette smoking [b]				
Yes	106(70.7)	82(54.7)	1.998(1.241-3.217)	0.0059
No	44(29.3)	68(45.3)		
Marital status [b]				
Married	112(74.7)	78(52.0)	2.721(1.670-4.432)	<0.0001
Unmarried	38(25.3)	72(48.0)		
Family history[b]				
Yes	123(82.0)	90(60.0)	3.046(1.793-5.176)	<0.0001
No	27(18.0)	60(40.0)		
First use of alcohol, age in years[C] (mean±SD)	20.07±5.57	20.09±4.16		0.8837

[a] Student's t test

[b] Chi square test

[c] Pearson correlation test

Table 2

Genotype and allele frequencies of *ADH1C* and *ALDH2* among case(n=150) and control (n=150) groups

SNP	Gene	Genotype	Case n(%)	Control n(%)	Allele	Case n(%)	Control n(%)
rs698	*ADH1C*	TT	57(38)	68(45)	T	184(61.3)	200(66.7)
		TC	70(47)	64(43)	C	116(38.7)	100(33.3)
		CC	23(15)	18(12)			
rs671	*ALDH2*	GG	73(49)	93(62)	G	209(69.7)	236(78.7)
		GA	63(42)	50(33)	A	91(30.3)	64(21.3)
		AA	14(09)	07(05)			

Table 3

Allelic and genotypic analysis of *ADH1C* and *ALDH2* among case and control groups

Models	P value	OR (95% CI)
rs698 (*ADH1C*)		
Dominant (TT Vs TC+CC)	0.2415	0.7391 (0.4733 - 1.179)
Recessive (CC Vs TT+ TC)	0.5018	1.328 (0.6700 - 2.588)
Co dominant (TC Vs CC+TT)	0.5615	1.176 (0.7433 - 1.867)
Additive (CC Vs TT)	0.2818	1.524 (0.7447 - 3.013)
Allelic (C Vs T)	0.2020	0.7931 (0.5664 - 1.106)
rs671 (*ALDH2*)		
Dominant (GG Vs GA+AA)	**0.0272**	0.5811 (0.3720 - 0.9224)
Recessive (AA Vs GG+GA)	0.1734	2.103 (0.8122 - 5.409)
Co dominant (GA Vs GG+AA)	0.1526	1.448 (0.9134 - 2.307)
Additive (GG Vs AA)	0.0634	0.3925 (0.1544 - 0.9832)
Allelic (G Vs A)	**0.0152**	0.6228 (0.4328 - 0.9009)

Pearson's chi-squared test

CI – confidence interval, OR – odds ratio, P value (<0.05) - significant