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DRD1 (-48A/G *Dde*I) gene polymorphism and alcohol dependence – a study among *Meiteis* of Manipur, India

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ABSTRACT

Dopamine receptors play a key role of the mesolimbic dopamine systems in reinforcing effects of alcohol and other drug abuse/addiction. The present study aims to assess the association of DRD1 gene polymorphism (-48 A/G DdeI) with alcohol dependence (AD). A case-controls study was carried out comparing 143 AD cases, who all met DSM-IV criteria for AD, and 318 age sex match controls from Meiteis of Manipur, India. Smoking status were SIGNIFICANTLY associated with AD (OR=2.983, 95% CI 1.970-4.517, p=0.000). Genotypic and allelic frequency distribution of -48A/G DdeI polymorphism did not differ significantly between the two groups. However, in recessive model mutant homozygous (GG) has shown an OR of 1.03, though not significant (95% CI=0.583-1.820; p=0.92). Interaction analysis of DRD1 -48A/G DdeI polymorphism and smoking status showed borderline significant increase risk for AD (OR=1.463, 95% CI 0.993-2.155, p=0.054). The present study suggests that -48A/G DdeI gene polymorphism is not a risk genetic marker for AD, though risk is likely to be increase among individuals possessing G allele and who smoked in the studied population.

Key Words: *Dopamine receptor, DRD1, alcohol, smoking, gene-environment interaction*

INTRODUCTION

Alcohol dependence (AD), a complex disorder, has become one of the most hazardous health problems which is reported to be mainly due to gene and environment interaction. Of the several genetic pathways implicated in the etiology of AD, dopaminergic system is one. Dopamine receptor genes are reported to be associated with various addictive disorders such

as alcohol dependence, drug abuse, movement disorders and mental health disorders like schizophrenia (Le Foll et al. 2009). It has been demonstrated that dopamine receptors play a key role of the mesolimbic dopamine systems in reinforcing effects of alcohol and other drug abuse/addiction (Nestler et al. 1993). Much attention has been paid to the gene coding for the dopamine D2 receptor (*DRD2*), since *Taq*IA1 has been reported to be associated with AD (Blum et al. 1990; Lawford et al. 1997; Young et al. 2004; Suraj Singh et al. 2013). The gene coding for dopamine D1 receptor (*DRD1*) has also been considered as a potential genetic marker in AD studies, particularly regarding its role within the prefrontal cortex in the modulation of cognitive processes (Rinaldi et al. 2007). A single nucleotide substitution A to G at position -48 (5` UTR) has been reported to be associated with AD with high sensation seeking scores (Comings et al.1997; Limosin et al. 2003). *DRD1* gene has also been found to involve in a number of brain functions including motor control, inattentive symptoms of attention deficit/hyperactive disorder and reward and reinforcement mechanisms (Misener et al. 2004; Luca et al. 2007). Thus, the present study attempts to understand the genetic etiology of AD among *Meiteis* of Manipur, a Mendelian population, having East Asian genetic ancestry with special reference to *DRD1* -48A/G *Dde*I gene polymorphism.

METHODS

The present study is carried out among males of the *Meitei* community, a Mendelian population from Manipur, India. A total of 461 blood samples were collected from individuals unrelated up to 1st cousin after obtaining pre-informed written consent. Of them, 143 were AD cases (mean age=49.68, range=25-72yrs) recruited either from rehabilitation centers or through household survey (who met DSM-IV criteria for alcohol dependence). 318 ethnic matched healthy controls (mean age=50.89, range=27-75yrs) were randomly recruited by household survey to control the ethnic differences. Data pertaining to personal information like age, smoking and alcohol consumption profile was also collected using interview schedule.

Genetic Analysis

Genomic DNA was extracted from the collected blood samples using standard protocol (Miller et al. 1988). Identification of *DRD1* -48A/G polymorphism was performed using previously described protocol using *Dde*I restriction enzyme digestion (Cichon et al. 1994).

Statistical Analysis

Genotype and allele frequencies were calculated by gene counting method and Hardy-Weinberg equilibrium was tested using POPGENE 1.31 (Yeh and Yang, 1999). Association of allelic and genotypic counts with AD cases were evaluated by Pearson's $\chi 2$ test followed by odds ratio (OR) at 95% confidential interval (CI) using freely available software (http://faculty.vassar.edu/lowry/odds2x2.html). Logistic regression analysis was also performed to look for association of the selected marker with AD, using the Windows SPSS version 15.0 software. Present study was approved by ethical committee of Department of Anthropology, University of Delhi.

RESULTS

In the present study, AD cases and their respective controls did not significantly differ with respect to age (t=1.18, p=0.238). The proportion of smoking individuals was found to be significantly higher among AD cases as compared to the controls ($p < 0.05$). Logistic regression analysis showed that smoking status was found to be significantly associated with AD (OR=2.983, 95% CI 1.970-4.517, p=0.000).

Table 1: Individual characteristics and distribution of genotypic and allelic frequency of *DRD1* -48A/G *Dde*I polymorphism among AD cases and controls

Characteristics		**Controls (N=318)**	**AD Cases (N=143)**	**(p-value)**
Age (year) (mean±SD)		50.89±10.04	49.68±10.44	t= 1.18 (0.238[a])
Smoking Status, n (%)	Smoke	79 (24.84)	71 (49.65)	$\chi 2$=27.7 (<0.001[b])
	Non-Smoke	239 (75.16)	72 (50.35)	
DRD1 -48A/G *Dde*I (rs4532)	GG	45 (14.15)	21 (14.69)	$\chi 2=0.92E^{-01}$ (0.96[b])
	AG	50 (15.72)	21 (14.69)	
	AA	223 (70.13)	101 (70.62)	
	G allele	0.22	0.22	$\chi 2=0.27E^{-04}$ (0.95[b])
	A allele	0.78	0.78	

[a]Unpaired *t*-test.
[b]Likelihood ratio $\chi 2$ test.

The *DRD1* gene was found to be polymorphic at the loci -48A/G in both AD cases and controls. Both AD cases and controls were found to deviate from Hardy Weinberg Equilibrium ($p<0.05$). Distribution of both wild and mutant homozygous genotype i.e. AA and GG respectively was found to be almost similar between AD cases and controls. No

significant difference was observed between them with respect to genotypic and allelic frequency distribution (p>0.05) (Table1).

In order to estimate the relative odds of a particular genotype (AG, GG or a combination of AG+GG) for alcohol dependence, odds ratio (OR) was calculated between AD cases and controls taking wild homozygous genotype i.e. AA as reference. Compared to the AA genotype, AG (dominant) and AG+GG genotypes (dominant and combination of dominant and recessive) exhibited no significant increased risk for alcohol dependence (OR=0.93, 95%CI=0.53-1.62, p=0.79 and OR=0.98, 95%CI=0.63-1.50, p=0.92 respectively). However, in recessive model mutant homozygous (GG) has shown an OR of 1.03, though not significant (95% CI=0.583-1.820; p=0.92). In order to understand the interaction of gene and environment, combined analysis was performed with respect to smoking status and *DRD1* -48A/G polymorphism. Individuals possessing G allele as well as who smoked showed 1.46 fold increased risk for AD. Though, the risk was found to be borderline significant (OR=1.463, 95% CI 0.993-2.155, p=0.054).

DISCUSSION

Few studies have been carried out for association of *DRD1* gene polymorphism and drug addictions, because of the absence of functional polymorphism identified. Available literatures indicate that expression of *DRD1* may be modulated by alcohol consumption which may further lead to susceptible for alcohol dependence (Sari et al. 2006).

In present study, no association was found between allele/genotype of *DRD1* -48A/G polymorphism with alcohol dependence among *Meiteis* of Manipur. This could possibly be due to the similar presentation of wild type (A) and mutant (G) allele among AD cases and controls. Individuals carrying G allele were found to be somewhat higher among the AD cases; however, the risk was not significant (OR = 1.03, 95% CI 0.583–1.820). This finding agrees with other similar association studies suggesting no role of this polymorphism in conferring susceptibility to alcohol dependence among different populations of world (Kim et al. 2007; Batel et al. 2008). Other independent studies demonstrated modest role of -48A/G *Dde*I polymorphism among alcohol addictive behaviours (Comings et al. 1997). Kim et al. observed no direct association of *DRD1* -48A/G polymorphism with AD cases among Korean population, however, shown positive association with severity of alcohol related problems (Kim et al. 2007). Similarly, independent risk of -48A/G polymorphism was not found with AD cases (Batel et al. 2008). Though, risk of alcohol dependence was higher among

individuals carrying a specific haplotype rs686*T-rs4532*G within the *DRD1* gene and also found to be significantly associated with AD cases (Batel et al. 2008). Moreover, association of this particular polymorphism was found with severe alcohol related problems such as high sensitive seeking scores (Limosin et al. 2003). Such inconsistent results could be attributed to different study designs, variation in sampling like selection of AD cases and nature of comparable controls besides differences in genetic structure of the populations.

On the other hand, risk of the G allele for AD is observed in interaction analysis, which shows a borderline significant increased risk of AD (OR=1.463, 95% CI 0.993-2.155, p=0.054). Findings of the present study supports the earlier reports suggesting involvement of *DRD1* in the locomotor and rewarding effects of drugs of abuse (Kalivas and Stewart, 1991). However, smaller sample size and consideration of single SNP in the present study could be major limitations for assessing association of *DRD1* -48A/G *Dde*I polymorphism with AD.

Conclusion

In brief, *DRD1* -48A/G *Dde*I polymorphism is not found to be associated with risk for AD in presently studied population. Though, individuals possessing G allele and who smoked may be at higher risk of AD. As AD is a complex behavioural trait which includes both genetic and environmental factors, one needs to focus on the role of *DRD1* -48A/G *Dde*I polymorphism in combination with other reported dopamine receptor gene polymorphisms and environmental factors. Results needs to be replicated and validated in population specific studies having common ethnic background in order to understand the complex etiology of alcohol dependence.

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