Association between Dopamine Gene and Alcoholism in Pategar Community of Dharwad, Karnataka

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Abstract: Alcoholic intoxication is a major social problem of public health all over the world. It is also related to a complex genetic association. The allele A9 of the dopamine transporter gene (DAT1; SLC6A3) was examined for association with alcoholism. The present study determines the distribution of the variable number of tandem repeat (VNTR) polymorphism in the 3'untranslated region of DAT1 in Pategar community of Dharwad. A group of 100 healthy controls (nonalcoholic) and 100 alcoholic patients were examined and genotyping study was done. The genotyping in individual gender was examined. The four allele frequencies 7, 9, 10 and 11 repeats of the DAT1 40-bp VNTR were detected. The analysis was carried out by using PCR and electrophoresis. The frequency of the allele A9 [f (A9+)] was significantly higher (P = 0.01) in the group of alcoholic patients [f (A9+) = 0.52] when compared with healthy controls (nonalcoholic) [f (A9+) = 0.30].The heterozygosity indices were low and varied from 0.090 to 0.390. This research study shows that the frequency of individuals carrying the allele A9 was significantly higher in the group of alcoholic patients compared with healthy controls. The results demonstrate the variability of the DAT1 40-bp VNTR polymorphism in Pategar community, Dharwad.

Index Terms: alcoholism, allele frequency, dopamine gene, dharwad, pategar community

I. INTRODUCTION

Alcoholic intoxication is a major social problem of public health all over the world. It is also related to a complex genetic association. Dopamine is a biogenicamine and is a key neurotransmitter in the brain tissue involvedin cardiovascular, renal, endocrinal and central nervoussystem regulation.The gene encoding DAT1SLC6A3 consistsof 15 exons,solute carrier family 6and spanning 60 kb on chromosome 5p15.32. Several studies have examined the associationsbetween variants in DAT1 gene and psychiatric disorders.The dopamine transporter gene (DAT) is essential for the regulation of dopaminergic neurotransmission. In most populations, A9 and A10 alleles repetition are common, although the A3, A5, A7, A8, A11 repeat alleles were also observed in various populations.This study is conducted to find out the association of allele A9 carrier status of DAT1 with a diagnosis of alcoholism in a case control design. The SLC6A3 genotypes in alcoholic patients show significant enhanced homozygote (genotypes 9/9 and 10/10) and reduced heterozygote (genotype 9/10) frequencies in contrast tohealthy controls.

Figure 1: Map of India showing the geographical location of the sampled area of the populations. The lines within the map indicate the borders of different states.

II. MATERIAL AND METHODS

The 200 individuals were selected fromPategar community of Dharwad and written informed consent was obtained from each of them.The screeningevaluations of all participating individuals were conducted by interview and clinical examination. Those were based on Structured Clinical Case Taking Proforma, MAST and AUDIT questionnaires. A group of 100 healthy controls (non-alcoholic) and 100 alcoholic patients were selected.Inclusion criteria for the healthy control group were: (1) absence of any neurological or psychiatric illnesses, (2) absence of pregnancy, (3) informed consent.Inclusion criteria for alcoholic patients were as follows: (1) diagnosis of alcoholic dependence (2) no history of severe medical conditions (3) absence of pregnancy (4) informed consent.

DNA isolation and genotyping:

The SDS- proteinase K method was used for extraction of DNA from a blood sample.

Statistical analysis:

The Fisher's exact test was used for comparison of allele A9 frequencies of the DAT 1 polymorphism between alcoholic patients and healthy controls.A significance level of 5% was considered for a type 1 error.Hot Water Distribution System Model program (HWSIM)and GENEPOP program were used for assessment of Hardy–Weinberg equilibrium (HWE) deviations.Using the FSTAT programcalculations of allele frequencies were observedand expected heterozygosity levels and F statistics were calculated.

III. RESULT AND DISCUSSIONS

All the 200 study subjects were genotyped for the DAT1 polymorphism. For all analyzed study subjects the genotype frequencies did not differ significantly from HWE ($P > 0.21$ in all cases). Table 1 exhibits the comparison between healthy controls and alcoholic patients on the basis of A9 allele. It is clear from the table that allele A9 was significantly ($P = 0.01$) higher in the group of alcoholic patients [f (A9+) = 0.52]when compared with healthy controls [f (A9+) = 0.30].

The male and female group exhibit variation in the expression of A9 allele; hence males and females wereanalyzedseparately and compared.Table 2 shows the distribution of A9 allele among male alcoholic patients and controls.It is clear from the table that, the frequency of the allele A9 was significantlyhigh [f (A9+) = 0.54] as compared to controls ($P = 0.02$).

Table 3 shows the distribution of A9 allele among female alcoholic patients and controls. The frequency of allele A9 issignificantly high [f (A9+) = 0.55] among alcoholic patients when compared to controls($P = 0.18$).

Table I:The Distribution of A9 alleles among the alcoholic patients andcontrols.

Sample	N	f(A9+)	P [f (A9+)]
Alcoholic patients	100	0.52	0.01
Healthy controls	100	0.30	

Table II: The comparison between male alcoholic patients and controls on the basis of A9 alleles.

Sample	N	f(A9+)	P[f (A9+)]
Malealcoholic patients	85	0.54	0.02
Malehealthy controls	75	0.32	

Table III. The comparison between female alcoholic patients and controls on the basis of A9 alleles.

Sample	N	f (A9+)	P[f (A9+)]
Female alcoholic patients	15	0.55	0.18
Female healthy controls	25	0.34	

Table IV: The Allele frequencies and Hardy – Weinberg proportions of the DAT1; SLC6A3 polymorphism in Pategar community of Dharwad.

Pategar community of Dharwad	Allele frequencies				HW X^2	*P* value
	7	9	10	11		
Alcoholic patients		0.250	0.720		7.177	0.066
Healthy controls	0.012	0.089	0.909		2.945	0.499

The frequency distribution of alleles is shown in Tables 4. This study clearly shows Hardy-Weinberg equilibrium and the analysis identified four alleles 7, 9, 10 and 11 -repeats. The 9 and 10-repeat alleles were more common in the Pategarcommunity. The frequency of 9 and 10-repeat allele is 0.250 and 0.720 in alcoholic patients. In healthy controls the frequency of 9 and 10-repeat allele is 0.089 and 0.909. The 11-repeat allele was not detected in the Pategar community. The 7-repeat allele was only observed in healthy controls with the frequency of 0.012.

Table V: The genotype frequencies and observed heterozygosity of the DAT1; SLC6A3 gene in Pategar community of Dharwad.

Pategar community of Dharwad	Genotype frequencies							Observed heterozygosity
	7/7	7/9	9/9	9/10	10/10	10/11	11/11	
Alcoholic patients			0.059	0.039	0.574			0.390
Healthy		0.032	0.090	0.890	0.710			0.090

controls								

Table 5 shows the distribution of the genotype frequencies and observed heterozygosity of the DAT1; SLC6A3 gene in the Pategar community. The heterozygosity indices were observed comparatively low in the healthy controls. These results demonstrate the variability of the DAT1 40-bp VNTR polymorphism in alcoholic patients and healthy controls among the Pategar community.

IV. CONCLUSIONS

It is clear from the present findings that there is an influence of DAT1 VNTR polymorphism in the etiology of alcoholism amongPategar community of Dharwad. This study also shows that most frequent nine and ten-repeat allele deviate significantly from alcoholic patients. The present research study establishes the association between allele A9 carrier status of DAT1 and alcoholism.

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