Lack of Association between a Functional Polymorphism in Dopamine and Opioid Receptor Genes with Alcoholism in South Indian Tamilian Population

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Single nucleotide polymorphism (SNP) of dopamine receptor (DRD2) and µ opioid receptor (OPRM1) genes are associated with susceptibility towards alcoholism. Hence, our study aimed to investigate the association of rs1799732 (DRD2) and rs1799971(OPRM1) with the risk of alcohol dependence in the south Indian Tamilian population and to compare the frequencies of these SNPs with major global populations. Methods: A total of 150 alcohol-dependent cases aged between 18 and 65 years who satisfied DSM-V were recruited from a de-addiction center (TTK hospital, Chennai). For the control group, 150 unrelated male blood donors with an AUDIT score of less than 8 were included. Genomic DNA was extracted and the alleles were genotyped using Taqman SNP genotyping assays by quantitative PCR. Results: Minor allele frequency (MAF) of rs1799732 and rs1799971 among controls were 16.7% and 50.3% respectively. Fisher’s exact test showed a highly significant difference in MAF of DRD2 and OPRM1 between south Indian controls and European as well as African populations. All the five genetic models of SNPs were evaluated using the Chi-square test revealed no statistically significant association between the SNPs and alcohol dependence. Conclusions: The present study did not find any association between DRD2 -141C Ins/Del and A118G OPRM1 polymorphism with alcoholism in the Tamilian population of south India.

Keywords: Alcohol Dependence; Association Study; Dopamine of Rs1799732; opioid of rs1799971; Polymorphism; Tamilian population.
Although the reward cascade is mainly formed by four important neurotransmission pathways viz., dopamine (DA), serotonin, opioid, and gamma-aminobutyric acid, the pleasure neurotransmitter dopamine plays a critical role in the initiation of alcohol use disorders. This is evident by the findings that alcohol administration increases DA levels in NAc. The reward model suggests that the endogenous opioid also plays a key role in the rewarding properties of alcohol by interacting with the dopamine receptor. Furthermore, heightened dopamine level following alcohol intake is secondary to the binding of endogenous opiates to the i opioid receptor. Hence, blockade or deficiency of dopamine reduces the rewarding effects leading to alcohol dependence (AD).

Previously, animal models were used to explore the underpinning mechanism of alcohol-induced dopamine release. Alcohol exerts its action primarily by enhancing neurotransmitter release resulting in higher dopamine levels in the synaptic cleft rather than by blocking the dopamine transporter. Therefore, any genetic variations in the functional regions of genes coding these targets result in gene dysfunction, probably associated with the development of alcoholism. Further literature search on alcoholism revealed that genetic variations might confer either protection or increase the risk of susceptibility towards the development of alcohol-related complications. 

The DRD2 gene located on postsynaptic dopaminergic neurons that are predominantly involved in reward signal transduction. Among dopamine receptor polymorphisms (Taq IA, B, and D), it is worth investigating -141C Ins/Del, rs1799732 which is a functional polymorphism located in the promoter region of DRD2 that involves insertion or deletion of cytosine. An in-vitro analysis reported that -141C Ins/Del polymorphism of DRD2 alters the transcriptional activity and thus plays an important role in regulating DRD2 expression. Further, it has been observed that the presence of the deletion allele reduces DRD2 expression by approximately 68% while the C insertion allele might cause dopaminergic hyperactivity. Regarding the association between rs1799732 and alcoholism, Mexican American and north Indian alcoholics showed a significant association of wild-type Ins allele with alcoholism whereas an association between mutant deletion variant and alcoholism is seen among German alcoholics.

The OPRM1 gene located at chromosome 6q25.2 encodes the 7-transmembrane G protein-coupled receptor is targeted by both endogenous and exogenous opioids. rs1799971 in exon 1 (118A>G) of OPRM1 is the most widely studied SNP in association with AD. This SNP results in an Asp40Asp amino acid change at a putative glycosylation site in the extracellular loop of the receptor increasing its ligand affinity. In cell culture, variant 118G binds beta-endorphins and activates G protein-coupled protein potassium ion channels with 3 times greater potency than receptors coded by the 118A variant. Individuals carrying the 118G allele show greater alcohol-induced striatal dopamine release than A/A homozygotes revealing the role of A118G SNP on the neurobiological response to alcohol. Despite many studies showing no association between rs1799971 and alcoholism, a study involving alcohol-dependent individuals from central Sweden revealed that the 118G allele is associated with an increased attributable risk for AD. Discrepancies in these association studies might be due to the inclusion of subjects from different ancestry or the contribution of different phenotypes.

The frequency distribution of SNPs varies across different populations and inter-ethnic genetic differences could be modifiers of clinical outcomes. Therefore, the present study aimed to investigate the possible association of rs1799732 and rs1799971 with alcoholism among the south Indian Tamilian population. The study was also designed to compare the frequencies of these SNPs with major global populations to explore any inter-ethnic differences.

**Methodology**

**Study population**

A total of 150 male alcohol-dependent subjects aged between 18-65 years were recruited from TTK hospital, a de-addiction center in Chennai. An expert psychiatrist for substance abuse assessed and examined the subjects for alcohol dependence as defined by DSM-V (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) for enrollment as cases. Blood donors who attended the blood bank of Tagore Medical College
and Hospital (TMCH) with a history of alcohol exposure were screened for harmful use of alcohol using the Alcohol Use Disorders Identification Test (AUDIT). About 150 unrelated healthy male donors aged 18–65 years with no pathological alcohol use (AUDIT score < 8) were further evaluated by DSM-V and recruited as a control sample for the study. Subjects in both groups with major psychiatric illnesses such as schizophrenia, depression bipolar disorder and other substance abuse disorder (except nicotine) were excluded. All the subjects had at least three generations of ancestors exclusively of south Indian ethnicity and also had the Tamil language as their mother tongue.

The study protocol was approved by the Institutional Ethics Committee, TMCH. Before commencement of the study, all the subjects were well informed about the procedure and written informed consent was obtained.

**Genomic DNA extraction and genotyping**

Under aseptic precautions, 2 ml of venous blood was collected and centrifuged (2500rpm for 10 minutes). Buffy coat was used to isolate DNA using the spin column-based DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) as per the manufacturer’s instructions. SNP genotyping for rs1799732 (assay ID: C_33641686_10) and rs1799971(assay ID: C_8950074_1_) was performed using 5’ exonuclease fluorescence TaqMan SNP genotyping assay kits (Applied Biosystems, Foster City, CA, USA) by real-time polymerase chain reaction. The assays were run on Bio-Rad CFX96 Real-Time PCR Detection System (Biorad Laboratories, CA, USA). The real-time PCR 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 the 90s. 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. Allelic discrimination was carried out using CFX Maestro Software 2.0 (Biorad Laboratories, CA, USA). The accuracy of genotyping was confirmed by randomly rerunning 15% of the samples.

**Statistical analysis**

Allele and genotype frequencies of rs1799732 and rs1799971 were calculated by the direct gene counting method. Genotype distributions of both the SNPs were tested for deviation from the Hardy-Weinberg equilibrium using the Chi-square test. Allelic and genotype frequencies of the SNPs were compared between alcohol dependent and control groups using Fisher’s exact test. Similarly, frequencies of both the SNPs from the control group of our study were compared with that of other global populations using Fisher’s exact test. The association of SNPs with alcohol dependence was evaluated by dominant, co-dominant, recessive, allelic, and additive genetic models using the Chi-square test followed by risk assessment using odds ratio (OR) and 95% confidence intervals (CIs). \( P < 0.05 \) was considered significant. Statistical analyses were performed using SPSS version 20 (IBM SPSS statistics NY, USA) and GraphPad Prism version 9 (San Diego, CA, USA).

**RESULTS**

The mean age (Mean ± SD) of alcohol-dependent subjects and controls was 37.69 ± 0.82 and 33.95 ± 1.09 years, respectively. The observed genotype frequency distributions of rs1799732 and rs1799971 were in agreement with Hardy Weinberg equilibrium (\( p > 0.05 \)). Allele and genotype frequencies of SNP in DRD2 and OPRM1 were shown in tables 1 and 2. On comparison, the allele and genotype frequencies showed no statistically significant difference between alcohol-dependent case and control groups (Table 1 and 2). The observed genotype and allele frequencies of the genetic variants in our study population have been compared with the established frequencies across major global populations (Table 3 and 4) using the data from the 1000 Genomes Project database. The highest and lowest minor allele frequency for the deletion variant of -141C DRD2 can be seen in Africans and Europeans respectively which is significantly different when compared to south Indian Tamilians. Moreover, the frequency of deletion variant in our control population is found similar to Americans and east Asians ranging between 14 to 16%. The study population showed the highest minor allele frequency for rs1799971(G allele) compared to the major global populations. Moreover, a highly significant statistical difference for the mutant G allele was observed between the south Indian...
Tamilians and in all four major global populations. Dominant, recessive, codominant, additive, and allelic genetic models of DRD2 and OPRM1 among alcohol-dependent cases and controls revealed no statistically significant association between the SNP and alcohol dependence as shown in table 5. 

**DISCUSSION**

The present study established the minor allele deletion variant of rs1799732 at a frequency of 16.7% among the Tamilian control group. The frequency of deletion variant is observed at

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Alcohol dependent % (n)</th>
<th>95% CI</th>
<th>Control % (n)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799732DRD2</td>
<td>Ins/Ins</td>
<td>74(111)</td>
<td>66.4-80.3</td>
<td>71(107)</td>
<td>63.6-77.9</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Ins/Del</td>
<td>23(34)</td>
<td>16.6-30.0</td>
<td>24(36)</td>
<td>17.8-31.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Del/Del</td>
<td>03(05)</td>
<td>1.43-7.56</td>
<td>05(07)</td>
<td>2.27-9.31</td>
<td></td>
</tr>
<tr>
<td>rs1799971OPRM1</td>
<td>AA</td>
<td>29(43)</td>
<td>22.0-36.3</td>
<td>26(39)</td>
<td>19.6-33.5</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>43(65)</td>
<td>35.6-51.3</td>
<td>47(71)</td>
<td>39.5-55.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>28(42)</td>
<td>21.4-35.6</td>
<td>27(40)</td>
<td>20.2-34.2</td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence Interval, N denotes sample size; n denotes number of genotypes. The p-values were obtained using Fisher’s exact test, P value (<0.05) considered as significant.

**Table 2. Comparison of allele frequencies among alcohol dependent (2N=300) and control (2N=300) groups**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Alcohol dependent n(%)</th>
<th>95%CI</th>
<th>Control n(%)</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799732DRD2</td>
<td>Ins</td>
<td>256(85.3)</td>
<td>80.8-88.8</td>
<td>250(83.3)</td>
<td>78.7-87.1</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Del</td>
<td>44(14.7)</td>
<td>11.1-19.1</td>
<td>50(16.7)</td>
<td>12.8-21.2</td>
<td></td>
</tr>
<tr>
<td>rs1799971OPRM1</td>
<td>A</td>
<td>151(50.3)</td>
<td>44.7-55.9</td>
<td>149(49.7)</td>
<td>44.0-55.2</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>149(49.7)</td>
<td>44.0-55.2</td>
<td>151(50.3)</td>
<td>44.7-55.9</td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence Interval, N denotes sample size; n denotes number of alleles. The p-values were obtained using Fisher’s exact test, P value (<0.05) considered as significant.

**Table 3. Comparison of genotype frequencies between study population (controls) and major global populations retrieved from the 1000 Genomes Project**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Tamilian (N=150)</th>
<th>African (N=661)</th>
<th>Frequency % American (N=347)</th>
<th>East Asian (N=504)</th>
<th>European (N=503)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799732</td>
<td>Ins/Ins</td>
<td>71.0(107)</td>
<td>18.6(123)***</td>
<td>70.9(246)***</td>
<td>74.8(377)***</td>
<td>83.5(420)*</td>
</tr>
<tr>
<td></td>
<td>Ins/Del</td>
<td>24.0(36)</td>
<td>48.7(322)</td>
<td>26.8(93)</td>
<td>23.0(116)</td>
<td>16.1(81)</td>
</tr>
<tr>
<td></td>
<td>Del/Del</td>
<td>05.0(07)</td>
<td>32.7(216)</td>
<td>2.3(08)</td>
<td>2.2(11)</td>
<td>0.4(02)</td>
</tr>
<tr>
<td>rs1799971</td>
<td>AG</td>
<td>47.0(71)</td>
<td>1.8(12)</td>
<td>30.8(107)</td>
<td>48.0(242)</td>
<td>27.2(137)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>27.0(40)</td>
<td>4.6(16)</td>
<td>15.3(77)</td>
<td>2.6(13)</td>
<td></td>
</tr>
</tbody>
</table>

N denotes sample size, values in parentheses indicate the number of genotypes
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS - Not significant, the p-values were obtained using Fisher’s exact test.
35% and 55% in the north (N=60) and central (N=50) Indian populations respectively which are reported to be higher when compared to our control population. However, their sample size is much smaller than the present study. The variant has been found to play a significant role in the etiology of early-onset alcohol dependence. In comparison with major global populations, MAF of rs1799732 in our study population is found to be almost similar to the frequencies in American (16%) and East Asian (14%) populations. However, a Korean study reported del variant at a frequency of about 36% and 27.3% among its healthy male and female populations respectively. On the other hand, the northern Chinese Han population observed del allele at the lowest frequency of 8.6% in healthy males which is quite similar to the frequency seen in the European population.

Table 4. Comparison of allele frequencies between study population (controls) and major global populations retrieved from the 1000 Genomes Project

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Tamilian (2N=300)</th>
<th>African (2N=1322)</th>
<th>Frequency %</th>
<th>American (2N=694)</th>
<th>East Asian (2N=1008)</th>
<th>European (2N=1006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799732</td>
<td>Ins</td>
<td>83.3(250)</td>
<td>43.0(568)***</td>
<td>84.0(585)***</td>
<td>86.0(870)***</td>
<td>92.0(921)***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Del</td>
<td>16.7(50)</td>
<td>57.0(754)</td>
<td>16.0(109)</td>
<td>14.0(138)</td>
<td>8.0(85)</td>
<td></td>
</tr>
<tr>
<td>rs179971</td>
<td>A</td>
<td>49.7(149)</td>
<td>99.1(1310)***</td>
<td>80.0(555)***</td>
<td>60.7(612)***</td>
<td>83.8(843)****</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>50.3(151)</td>
<td>0.9(12)</td>
<td>20.0(139)</td>
<td>39.3(396)</td>
<td>16.2(163)</td>
<td></td>
</tr>
</tbody>
</table>

N denotes sample size, values in parentheses indicate the number of alleles.
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS - Not significant, the p-values were obtained using Fisher’s exact test.

Table 5. Allelic and genotypic analysis of the SNP in DRD2 and OPRM1 among alcohol dependent and control groups

<table>
<thead>
<tr>
<th>Genetic models</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799732 (DRD2) Dominant (Ins/Ins Vs Ins/del+del/del)</td>
<td>1.144 (0.6905 - 1.908)</td>
<td>0.69</td>
</tr>
<tr>
<td>Recessive (del/del Vs del/Ins +Ins/Ins)</td>
<td>0.7044 (0.2490 - 2.067)</td>
<td>0.76</td>
</tr>
<tr>
<td>Co dominant (Ins/del Vs del/del+Ins/Ins)</td>
<td>0.9282 (0.5466 - 1.569)</td>
<td>0.89</td>
</tr>
<tr>
<td>Additive (Ins/Ins Vs del/del)</td>
<td>1.452 (0.4884 - 4.131)</td>
<td>0.56</td>
</tr>
<tr>
<td>Allelic Ins Vs del rs179971 (OPRM1) Dominant (AA Vs AG+GG)</td>
<td>1.144 (0.6905 - 1.908)</td>
<td>0.69</td>
</tr>
<tr>
<td>Recessive (GG Vs AA+ AG)</td>
<td>1.069 (0.6450 - 1.779)</td>
<td>0.89</td>
</tr>
<tr>
<td>Co dominant (AG Vs AA+ GG)</td>
<td>0.8509 (0.5366 - 1.344)</td>
<td>0.56</td>
</tr>
<tr>
<td>Additive (AA Vs GG)</td>
<td>1.050 (0.5780 - 1.891)</td>
<td>0.99</td>
</tr>
<tr>
<td>Allelic (A Vs G)</td>
<td>1.027 (0.7456 - 1.415)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Pearson’s Chi-squared test, CI – confidence interval, OR – odds ratio, P value (<0.05) considered as significant.
In our study, the frequency of the -141C Ins allele was found to be higher in alcohol-dependent subjects when compared to controls. However, there were no statistically significant differences in allele frequency between these groups. In a case-control study conducted among the Japanese population there was a statistically significant increase in Ins allele frequency among alcoholics and the investigators also found an association between -141C Ins allele and alcoholism. A similar association between Ins allele and alcoholism was found with Mexican Americans, north Indian and Korean alcoholics. Lee et al suggested that the -141C Ins allele might be a predisposing factor for alcohol dependence in the Korean population as the polymorphism is related to low dopamine availability. Prasad et al explained that the craving reward pathway is stimulated due to decreased DRD2 receptor density in alcoholics which predisposes them to develop alcohol dependence. Further, the study suggested that -141C Ins allele carriers are at higher risk for developing AD than -141C del carriers, and the presence of -141C del/del genotype confers protection from AD among north Indian alcoholics. Since the power of the study was observed to be moderate, no definite conclusion can be drawn from the study.

On the contrary, another Indian study conducted among the Meitei community from Manipur belonging to east Asian ancestry found to have increased frequency of del allele among alcoholics. Moreover, the study failed to detect a statistically significant association between del allele and alcohol dependence even though the power of sample size was more than 90%. Since, there exists slight significance (P=0.059) between del allele and AD, the study concluded that the individuals with del allele are likely to have an increased risk of AD in the presence of Taq1A polymorphism of DRD2. Meanwhile, the frequency of the mutant del allele was reported to be significantly higher among German alcoholics with a family history of alcoholism and without a history of withdrawal symptoms. Further, the study suggested that del variant would be a risk factor in alcoholics with a paternal and grandpaternal history of alcoholism although the variant might be a protective factor against the development of withdrawal symptoms. The findings of our study demonstrated that no association was found between either Ins or del variant of rs1799732 and alcoholism which coincides with a study that investigated 74 alcohol-dependent Caucasian men with or without a genetic predisposition for alcoholism. Several studies across many ethnic populations found rs1799732 did not contribute a significant role in conferring vulnerability to alcoholism.

The present study documented the highest frequency for the G allele at 50.3% in the control group. Among major global populations, the mutant G allele is widely prevalent across all populations except Africans (0.9%). Case-control studies in other global populations on alcoholism revealed that G allele frequency was lower among control populations in Swedish (11%) and German populations (12%) and various Indian sub-populations. These findings suggest that the Indian genetic architecture is diverse and unique. An earlier study on the origins of the Indian population demonstrated that ancestral south Indians (ASI) are genetically distinct from ancestral North Indians (ANI) as well as East Asians.

The functional polymorphism rs1799971 resulting in a substitution of adenine to guanine at 118th position has gained popularity for affecting the peptide sequence of opioid receptor related to alcoholism. Findings from previous literature support the association between A118G polymorphism and alcohol rewarding effects. For instance, individuals carrying the 118G allele reported having a positive family history of AD and experiencing heightened responses like mood levels, sedation after alcohol intake as compared to controls. Though rs1799971 was shown to contribute in the development of alcohol addiction, our study findings reported no association which was in accordance with a recent meta-analysis involving 17 case-control studies. This meta-analysis were performed among Asian populations.
and Caucasian populations concluded that no association exists between AD and A118G SNP in either ethnicity. However, previous meta-analysis includes five Asian and seven Caucasian studies reported that rs1799971 may contribute to the susceptibility of alcohol dependence in Asians but not in Caucasians. Among the Asian population, Deb et al reported that the 118G allele was found to be associated with alcoholism among the Bengali population. Furthermore, the study found a higher prevalence of minor allele among alcoholics (39.6%) when compared to controls (28%). A similar association has been observed in the Japanese population with MAF at 52% and 43% among alcoholics (N=64) and controls (N=73) respectively. On the contrary, a case control study conducted in Korean alcoholics failed to detect significant association (p=0.105) even though homozygous (G/G) were shown to be associated with more drinking days than heterozygous (A/G) patients. Moreover, a recent epidemiological study using a large sample (n=965) of European ancestry found no relationship between rs1799971 and alcohol consumption. In addition, several meta-analyses of candidate gene studies and genome-wide association studies have shown no significant association between rs1799971 and AD which is in line with our study results.

These conflicting results from various genetic association studies between and within ethnic groups can be explained by the possible role of nongenetic factors like socio-cultural, religious, geographical, linguistic demarcations and endogamy practices implicated in the development of alcoholism. The study has investigated only one well-documented SNP in each of the candidate genes associated with alcoholism. Future studies could evaluate the combined effect of other significant SNPs modulating the clinical outcome reported in these genes with environmental or social factors, that may provide insights about the development of alcoholism and could support in its effective management.

**CONCLUSION**

The present study established the allele and genotype frequencies of rs1799732 and rs1799971 in the Tamilian population of south India. Significant differences and similarities in these frequencies were observed between the control population of our study and the major global populations. However, a lack of statistically significant association between -141C Ins/Del of DRD2 and A118G of OPRM1 and alcohol dependence has been observed in our study population.

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**Conflict of Interest**

There is no conflict of interest.

**REFERENCES**


