Biomedical & Pharmacology Journal, March 2023. Vol. **16**(1), p. 345-353

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

Umamageswari Arunachalam[1]*, Adithan Chandrasekaran[2] and Iyanar Kannan[1]

[1]Department of Pharmacology, Tagore Medical College and Hospital, Rathinamangalam, Vandalur, Chennai, Tamilnadu, India.
[2]Former Dean Research, Sri Balaji Vidyapeet, Puducherry, India.
*Corresponding Author E-mail: leeuma16@tagoremch.com

https://dx.doi.org/10.13005/bpj/2616

(Received: 21 January 2022; accepted: 21 November 2022)

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.

Alcoholism is a polygenic complex disorder, which is moderated by biochemical, metabolic, genetic, and neurological factors[1]. Among these, genetic factors for alcoholism are widely studied by the candidate gene association approach[2]. Genes in the mesolimbic reward system are considered to be the key neural substrate for alcohol-associated behaviors[3]. Hence, the genetic variants have gained the utmost importance and are frequently selected as candidates in these association studies.

In the brain, the mesolimbic dopaminergic pathway originates in the ventral tegmental area of the midbrain and extends to the nucleus accumbens (NAc), with connections further extending to the limbic system and the orbitofrontal cortex[4].

This is an Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).
Published by Oriental Scientific Publishing Company © 2023

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[5]. This is evident by the findings that alcohol administration increases DA levels in NAc[6]. 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[7]. Furthermore, heightened dopamine level following alcohol intake is secondary to the binding of endogenous opiates to the ì opioid receptor. Hence, blockade or deficiency of dopamine reduces the rewarding effects leading to alcohol dependence (AD) [8].

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[9]. Therefore, any genetic variations in the functional regions of genes coding these targets result in gene dysfunction, probably associated with the development of alcoholism[10,11]. 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[12,13].

DRD2 is a G protein-coupled receptor located on postsynaptic dopaminergic neurons that are predominantly involved in reward signal transduction[14]. 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[2]. 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[15]. 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[1]. Regarding the association between rs1799732 and alcoholism, Mexican American and north Indian alcoholics showed a significant association of wild-type Ins allele with alcoholism[16,17] whereas an association between mutant deletion variant and alcoholism is seen among German alcoholics[18].

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[19]. 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[20]. 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 [21]. Individuals carrying the118G allele show greater alcohol-induced striatal dopamine release than A/A homozygotes revealing the role of A118G SNP on the neurobiological response to alcohol[22.] 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[23]. 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 [24]. 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 ìL of the GoTaq® Probe qPCR Master Mix 2X (Promega Corporation, USA), 0.5 ìL TaqMan assay 40X, and 2.5ìL 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 1. Comparison of genotype frequencies among alcohol dependent (N=150) and control (N=150) groups

SNP	Genotype	alcohol dependent %(n)	95% CI	Control %(n)	95% CI	P value
rs1799732*DRD2*	Ins/Ins	74(111)	66.4-80.3	71(107)	63.6-77.9	0.78
	Ins/Del	23(34)	16.6-30.0	24(36)	17.8-31.4	
	Del/Del	03(05)	1.43-7.56	05(07)	2.27-9.31	
rs1799971*OPRM1*	AA	29(43)	22.0-36.3	26(39)	19.6-33.5	0.57
	AG	43(65)	35.6-51.3	47(71)	39.5-55.2	
	GG	28(42)	21.4-35.6	27(40)	20.2-34.2	

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

SNP	Allele	alcohol dependent n(%)	95%CI	Control n(%)	95%CI	P value
rs1799732*DRD2*	Ins	256(85.3)	80.8-88.8	250(83.3)	78.7-87.1	0.57
	Del	44(14.7)	11.1-19.1	50(16.7)	12.8-21.2	
rs1799971*OPRM1*	A	151(50.3)	44.7-55.9	149(49.7)	44.0-55.2	0.93
	G	149(49.7)	44.0-55.2	151(50.3)	44.7-55.9	

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

SNP	Genotype	Frequency % Tamilian (N=150)	African (N=661)	American (N=347)	East Asian (N=504)	European (N=503)
	Ins/Ins	71.0(107)	18.6(123)****	70.9(246)^{NS}	74.8(377)^{NS}	83.5(420)*
rs1799732	Ins/Del	24.0(36)	48.7(322)	26.8(93)	23.0(116)	16.1(81)
	Del/Del	05.0(07)	32.7(216)	2.3(08)	2.2(11)	0.4(02)
	AA	26.0(39)	98.2(649)****	64.6(224)****	36.7(185)^{NS}	70.2(353)****
rs1799971	AG	47.0 (71)	1.8(12)	30.8(107)	48.0(242)	27.2(137)
	GG	27.0(40)	0	4.6(16)	15.3(77)	2.6(13)

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[17,25]. 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[1]. 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[26]. However, a Korean study reported del variant at a frequency of about 36% and 27.3% among its healthy male and female populations respectively [27]. 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[28].

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

SNP	Allele	Frequency %				
		Tamilian (2N=300)	African (2N=1322)	American (2N=694)	East Asian (2N=1008)	European (2N=1006)
rs1799732	Ins	83.3(250)	43.0(568)****	84.0(585)^{NS}	86.0(870)^{NS}	92.0(921)***
	Del	16.7(50)	57.0(754)	16.0(109)	14.0(138)	8.0(85)
rs1799971	A	49.7(149)	99.1(1310)****	80.0(555)****	60.7(612)***	83.8(843)****
	G	50.3(151)	0.9(12)	20.0(139)	39.3(396)	16.2(163)

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

Genetic models	OR (95% CI)	P value
rs1799732 (*DRD2*)		
Dominant (Ins/Ins Vs Ins/del+del/del)	1.144 (0.6905 - 1.908)	0.69
Recessive (del/del Vs del/Ins +Ins/Ins)	0.7044 (0.2490 - 2.067)	0.76
Co dominant (Ins/del Vs del/del+Ins/Ins)	0.9282 (0.5466 - 1.569)	0.89
Additive (Ins/Ins Vs del/del)	1.452 (0.4884 - 4.131)	0.56
Allelic Ins Vs del	1.164 (0.7568 - 1.805)	0.57
rs1799971(*OPRM1*)		
Dominant (AA Vs AG+GG)	1.144 (0.6905 - 1.908)	0.69
Recessive (GG Vs AA+ AG)	1.069 (0.6450 - 1.779)	0.89
Co dominant (AG Vs AA+ GG)	0.8509 (0.5366 - 1.344)	0.56
Additive (AA Vs GG)	1.050 (0.5780 - 1.891)	0.99
Allelic (A Vs G)	1.027 (0.7456 - 1.415)	0.93

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 [29]. A similar association between Ins allele and alcoholism was found with Mexican Americans, north Indian and Korean alcoholics[16,17,30]. Lee et al suggested that the "141C Ins might be a predisposing factor for alcohol dependence in the Korean population as the polymorphism is related to low dopamine availability [30]. 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[17]. 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(62%), 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[3]. 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[18]. 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 [31]. Several studies across many ethnic populations found rs1799732 did not contribute a significant role in conferring vulnerability to alcoholism [32-34].

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%) [23,35]. Among the different Asian healthy populations, the G allele was observed at 31%, 35%, and 43% in Korean, Chinese, and Malaysians respectively[36]. In a north Indian (N=156) and West Bengal (N=82) control populations, the G allele was observed at 12% and 28% respectively[37,38]. The study demonstrated significant inter-ethnic differences in the prevalence of rs1799971 with other global populations as well as various Indian sub-populations. These findings suggest that the Indian genetic architecture is diverse and unique[39]. 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 [40].

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 [41]. 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[42]. 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[43]. This meta-analysis were performed among Asian

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[44]. Among the Asian population, Deb et al reported that the 118G allele was found to be associated with alcoholism among the Bengali population[38]. 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[45]. 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[46]. 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[47-50].

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 (40). 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.

ACKNOWLEDGEMENT

This study had received a research grant from The Tamil nadu Dr. MGR Medical University, Chennai, India.

Conflict of Interest

There is no conflict of interest.

REFERENCES

1. Grzywacz A, Jasiewicz A, Ma³ecka I,Suchanecka A, Grochans E, Karakiewicz B, et al. Influence of DRD2 and ANKK1 polymorphisms on the manifestation of withdrawal syndrome symptoms in alcohol addiction. Pharmacol Rep.2012;64(5):1126-34.
2. Du Y, Wan YJ. The interaction of reward genes with environmental factors in contribution to alcoholism in mexican americans. Alcohol Clin Exp Res.2009;33(12):2103-12.
3. Suraj Singh H, Ghosh PK, Saraswathy KN. DRD2 and ANKK1 gene polymorphisms and alcohol dependence: a case-control study among a Mendelian population of East Asian ancestry. Alcohol Alcohol.2013;48(4):409-14.
4. Fedorenko OY, Mikhalitskaya EV, Toshchakova VA, Loonen AJM, Bokhan NA, Ivanova SA. Association of *PIP4K2A* polymorphisms with alcohol use disorder. Genes (Basel).2021;12(10):1642.
5. Blum K, Bailey J, Gonzalez AM, Oscar-Berman M, Liu Y, Giordano, et al. Neuro-genetics of reward deficiency syndrome (RDS) as the root cause of "Addiction Transfer": A new phenomenon common after Bariatric surgery. J Genet Syndr Gene Ther.2011;2012(1):S2-001.
6. Ma H, Zhu G. The dopamine system and alcohol dependence.Shanghai Arch Psychiatry.2014;26(2):61-8.
7. Popova D, Desai N, Blendy JA, Pang ZP. Synaptic regulation by OPRM1 variants in reward neurocircuitry. J Neurosci.2019;39(29):5685-96.
8. Le Foll B, Gallo A, Le Strat Y, Lu L, Gorwood P.Genetics of dopamine receptors and drug addiction: a comprehensive review. Behav Pharmacol.2009;20(1):1-17.

9. Yim HJ, Gonzales RA. Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol.2000;22(2):107-15.
10. Thorsell A. The ì-opioid receptor and treatment response to naltrexone. Alcohol. Alcohol.2013;48(4):402-8.
11. Burns JA, Kroll DS, Feldman DE, Kure Liu C, Manza P, Wiers CE, et al. Molecular imaging of opioid and dopamine systems: Insights into the pharmacogenetics of opioid use disorders.Front Psychiatry.2019;10:626.
12. Long JC, Knowler WC, Hanson RL,Robin RW, Urbanek M, Moore E, et al. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. Am J Med Genet.1998;81(3):216-21.
13. Saccone NL, Kwon JM, Corbett J, Goate A,Rochberg N, Edenberg HJ, et al. A genome screen of maximum number of drinks as an alcoholism phenotype. Am J Med Genet. 2000;96(5):632-37.
14. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat. 2004;23(6):540-5.
15. Arinami T, Gao M, Hamaguchi H,Toru M. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet.1997;6(4):577-82.
16. Konishi T, Luo HR, Calvillo M, Mayo MS, Lin KM, Wan YJ. ADH1B*1, ADH1C*2, DRD2 (-141C Ins), and 5-HTTLPR are associated with alcoholism in Mexican American men living in Los Angeles. Alcohol Clin Exp Res.2004;28(8):1145-2.
17. Prasad P, Ambekar A, Vaswani M. Dopamine D2 receptor polymorphisms and susceptibility to alcohol dependence in Indian males: a preliminary study. BMC Med Genet.2010;11:24.
18. Johann M, Putzhammer A, Eichhammer P,Wodarz N. Association of the -141C Del variant of the dopamine D2 receptor (DRD2) with positive family history and suicidality in German alcoholics. Am J Med Genet B Neuropsychiatr Genet.2005;132B(1):46-9.
19. Kim KM, Choi SW, Kim D, Lee J, Kim JW. Associations among the opioid receptor gene (*OPRM1*) A118G polymorphism, psychiatric symptoms, and quantitative EEG in Korean males with gambling disorder: A pilot study.J Behav Addict.2019;8(3):463-70.
20. Mansour Shakiba, Mohammad Hashemi, Zahra Rahbari, Salah mahdar, Hiva Danesh, Fatemeh Bizhani, et al. Lack of association between Human ì- Opioid receptor (OPRM1) gene polymorphisms and Heroin addiction in a sample of southeast Iranian population. AIMS Medical Science.2017;4(2):233-40.
21. Hartwell EE, Feinn R, Morris PE, Gelernter J, Krystal J, Arias AJ, et al. Systematic review and meta-analysis of the moderating effect of rs1799971 in OPRM1, the mu-opioid receptor gene, on response to naltrexone treatment of alcohol use disorder. Addiction. 2020;115(8):1426-37.
22. Herz A. Endogenous opioid systems and alcohol addiction.Psychopharmacology., 1997;129:99–111.
23. Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, et al. Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. Neuropsycho pharmacology.2005;30(2):417-22.
24. Scarnati MS, Boreland AJ, Joel M, Hart RP, Pang ZP. Differential sensitivity of human neurons carrying ì opioid receptor (MOR) N40D variants in response to ethanol. Alcohol.2020;87:97-109.
25. Moumita Sinha, Niketa Vishwakarma, Ashish Pradhan, Arjun Rao I, Bharti Ahirwar, Mitashree Mitra.The role of Dopamine D2 receptor polymorphism towards susceptibility to alcohol dependence among male cases of Bilaspur district: A preliminary study.EJBPS.2017;4(12):851-7.
26. Ensembl database. Population genetics.https://asia.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=11:113475030-113476030;v=rs1799732;vdb=variation;vf=165588878. Accessed August 30, 2021.
27. Paik SH, Choi MR, Kwak SM, Bang SH, Chun JW, Kim JY, et al. An association study of Taq1A ANKK1 and C957T and - 141C DRD2 polymorphisms in adults with internet gaming disorder: a pilot study. Ann Gen Psychiatry.2017;16:45.
28. Zhang XC, Ding M, Adnan A, Liu Y, Liu YP, Xing JX, et al. No association between polymorphisms in the promoter region of dopamine receptor D2 gene and schizophrenia in the northern Chinese Han population: A case-control study. Brain Behav.2019;9(2):e01193.
29. Ishiguro H, Arinami T, Saito T, Akazawa S, Enomoto M, Mitushio H, et al. Association study between the -141C Ins/Del and TaqI A polymorphisms of the dopamine D2 receptor gene and alcoholism. Alcohol Clin Exp Res.1998;22(4):845-8.

30. Lee SH, Lee BH, Lee JS,Chai YG, Choi MR, Han DM, et al. The association of DRD2 -141C and ANKK1 TaqIA polymorphisms with alcohol dependence in Korean population classified by the Lesch typology. Alcohol Alcohol.2013;48(4):426-32.
31. Wiesbeck GA, Dürsteler-MacFarland KM, Wurst FM,Walter M, Petitjean S, Müller S, et al. No association of dopamine receptor sensitivity in vivo with genetic predisposition for alcoholism and DRD2/DRD3 gene polymorphisms in alcohol dependence. Addict Biol.2006;11(1):72-5.
32. Chen WJ, Chen CH, Huang J, Hsu YP, Seow SV, Chen CC, et al. Genetic polymorphisms of the promoter region of dopamine D2 receptor and dopamine transporter genes and alcoholism among four aboriginal groups and Han Chinese in Taiwan. Psychiatr Genet.2001;11(4):187-95.
33. Parsian A, Cloninger CR, Zhang ZH. Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am J Med Genet.2000;96(3):407-11.
34. Sander T, Ladehoff M, Samochowiec J, Finckh U, Rommelspacher H, Schmidt LG. Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. Alcohol Clin Exp Res.1999;23(4):578-81.
35. Franke P, Wang T, Nöthen MM, Knapp M, Neidt H, Albrecht S, et al. Nonreplication of association between mu-opioid-receptor gene (*OPRM1*) A118G polymorphism and substance dependence. Am J Med Genet.2001;105(1):114-9.
36. Berrettini W. Opioid pharmacogenetics of alcohol addiction. Cold Spring Harb Perspect Med.2013;3(7):a012203.
37. Kapur S, Sharad S, Singh RA,Gupta AK. A118G polymorphism in mu opioid receptor gene (OPRM1): association with opiate addiction in subjects of Indian origin. J Integr Neurosci.2007;6(4):511-22.
38. Deb I, Chakraborty J, Gangopadhyay PK, Choudhury SR, Das S. Single-nucleotide polymorphism (A118G) in exon 1 of *OPRM1* gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. J Neurochem.2010;112(2):486-96.
39. Tamang R, Singh L, Thangaraj K. Complex genetic origin of Indian populations and its implications.J Biosci.2012;37(5):911-9.
40. Reich D, Thangaraj K, Patterson N,Price AL, Singh L. Reconstructing Indian population history.Nature.2009;461(7263):489-94.
41. Gurel SC, Ayhan Y, Karaaslan C, Akel H, Karaca RÖ, Babaoðlu MÖ et al. Mu-opioid receptor gene (OPRM1) polymorphisms A118G and C17T in alcohol dependence: a Turkish sample.Turk Psikiyatri Derg.2016;27(2):1-8.
42. Ray LA, Hutchison KE. A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. Alcohol Clin Exp Res.2004;28(12):1789-95.
43. Kong X, Deng H, Gong S, Alston T, Kong Y, Wang J. Lack of associations of the opioid receptor mu 1 (OPRM1) A118G polymorphism (rs1799971) with alcohol dependence: review and meta-analysis of retrospective controlled studies. BMC Med Genet.2017;18(1):120.
44. Chen D, Liu L, Xiao Y, Peng Y, Yang C, Wang Z. Ethnic-specific meta-analyses of association between the OPRM1 A118G polymorphism and alcohol dependence among Asians and Caucasians. Drug Alcohol Depend.2012;123(1-3):1-6.
45. Nishizawa D, Han W, Hasegawa J, IshidaT, Numata Y, Sato T, et al. Association of mu-opioid receptor gene polymorphism A118G with alcohol dependence in a Japanese population. Neuropsy chobiology.2006;53(3):137-41.
46. Sloan ME, Klepp TD, Gowin JL, Swan JE, Sun H, Stangl BL, et al. The OPRM1 A118G polymorphism: converging evidence against associations with alcohol sensitivity and consumption. Neuropsycho pharmacology.2018;43(7):1530-8.
47. Schwantes-An TH, Zhang J, Chen LS, Hartz SM, Culverhouse RC, Chen X, et al. Association of the OPRM1 variant rs1799971 (A118G) with non-specific liability to substance dependence in a collaborative de novo meta-analysis of European-ancestry cohorts. Behav Genet. 2016;46(2):151-69.
48. Loh el W, Fann CS, Chang YT, Chang CJ, Cheng AT. Endogenous opioid receptor genes and alcohol dependence among Taiwanese Han. Alcohol Clin Exp Res.2004;28(1):15-9.
49. Pieters S, van Der Vorst H, Burk WJ, Schoenmakers TM, van der Willenberg E, Smeets HJ, et al. The effect of the *OPRM1* and *DRD4* polymorphisms on the relation between attentional bias and alcohol use in adolescence and young adulthood. Dev Cogn Neurosci.2011;1(4):591–9.
50. Koller G, Zill P, Rujescu D, Ridinger M, Pogarell O, Fehr C, et al. Possible association between *OPRM1* genetic variance at the 118 locus and alcohol dependence in a large treatment sample: relationship to alcohol dependence symptoms. Alcohol Clin Exp Res.2012;36(7):1230–6.