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# An Association Study of the COMT and GABA Gene Variants with Alcohol Dependence

**Renu Singh**[1], **Tripti Grover**[1], **Ranjan Gupta**[1], **Atul Ambekar**[2], **Raka Jain**[2] **and Arundhati Sharma**[1*]

[1]*Laboratory of Cyto-Molecular Genetics, Department of Anatomy, AIIMS, New Delhi, India*

[2]*National Drug Dependence Treatment Center, Department of Psychiatry, AIIMS, New Delhi, India*

[*]**Corresponding author:** Arundhati Sharma, Laboratory of Cyto-Molecular Genetics, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi, India, Tel: 01126593489; Fax: 01126588626; E-mail: arundhatisharma1@gmail.com

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## Abstract

Alcohol dependence (AD), associated with high mortality and morbidity is caused due to involvement of both environmental and genetic factors. Behavioral effects of alcohol including cognitive impairment, motor incoordination, tolerance and dependence are likely due to its effect on the multiple brain proteins including neurotransmitters. Dysfunction in these neurotransmitter systems may be at the level of enzymes involved in metabolic degradation, or receptors involved in neurotransmission like dopamine, Gamma-amino butyric acid (GABA), serotonin etc. Genetic polymorphisms in these neurotransmitter systems are implicated in conferring susceptibility to AD.

**Aim:** To identify association of single nucleotide polymorphisms (SNPs) in COMT (rs4680 and rs2075507) and GABAA receptor genes (rs13172914 and rs211014) with AD.

**Method:** A total of 100 AD patients diagnosed on the basis of DSM IV criteria from the outpatient clinic of the National Drug Dependence Treatment Centre (NDDTC) of All India Institute of Medical Sciences (AIIMS), and 100 healthy individuals from the general population were recruited. A detailed history including pattern of drug use and demographic details with pedigree information was noted. Genomic DNA from peripheral blood samples was processed for PCR amplification followed by restriction digestion to screen for the presence of polymorphisms. Genotype and allele frequencies were evaluated and correlated with alcohol use parameters including duration of alcohol use, age at onset of alcohol use, quantity of alcohol consumed (gms/day) and WHO ASSIST score and levels of liver function enzymes (SGPT and SGOT). Statistical analysis was performed using SPSS v21.0.

**Results:** Genetic analysis of the study group revealed COMT rs4680 to be significantly associated with AD (p=0.03), while the other COMT SNP rs2075507 showed an association with increased levels of SGPT in the patients. GABAA receptor gene polymorphisms showed no association with AD.

**Conclusion:** The study suggests a role of COMT gene polymorphism rs4680 in conferring susceptibility to AD.

**Keywords:** Alcohol dependence; Gene; Polymorphisms; GABAergic pathway; Dopaminergic pathway; COMT

## Introduction

Alcohol dependence (AD) has emerged as a major social and health problem globally and is responsible for high mortality and morbidity [1]. Several studies report on the genetic predisposition to alcohol dependence, although this does not deny the role of environmental factors [1-3]. Literature also suggests the presence of SNPs in the reward pathway genes (dopamine) as factors responsible for altered alcohol consumption. Apart from this, the serotonin, Gamma-amino butyric acid (GABA) and glutamate gene pathway polymorphisms are also known to play a role in AD.

Metabolic degradation of catecholamines such as dopamine, epinephrine, norepinephrine is an essential step in the regulation of neurotransmitters with specific enzymes contributing to the degradation process. The Catechol-o-methyl transferase enzyme encoded by the COMT gene is involved in dopamine degradation [4,5]. This enzyme catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines. This O-methylation is one of the major degradative pathways of the endogeneous catecholamine transmitters. COMT also has an important role in the metabolism of catechol drugs used in the treatment of hypertension, asthma and Parkinson disease. COMT is present in either membrane bound form or in soluble form in peripheral tissues including liver and blood. While the soluble form is postulated to play a role in the detoxification and metabolism of catechols, the membrane bound form is majorly involved in dopamine degradation [6]. Several polymorphisms in the COMT gene have been reported. One of the functional polymorphisms Val158Met (rs4680) is responsible for differential activity of this enzyme. This polymorphism leads to transition from G (Valine) to A (Methionine) at position 158 of COMT gene. COMT Val158 responsible for high activity of enzyme, results in lower pre-frontal dopamine signaling. This impaired pre-frontal cortical function may contribute to an increased risk of psychoactive disorders [7]. Polymorphisms in this gene have also shown strong association with

neuropsychiatric disorders including AD and heroin dependence [8-12].

GABA, an inhibitory neurotransmitter in the mammalian central nervous system affects various physiological and psychological processes. The GABA receptors, known to be involved in the development of neuropsychiatric disorders, exert their effects via the ionotropic ($GABA_A$) type A receptors which are ligand-gated chloride-channel complexes and metabotropic ($GABA_B$) type B receptors which are G protein coupled receptors [13]. $GABA_A$ receptors are a major target of alcohol action and drugs including benzodiazepines, barbiturates and anesthetic steroids [14]. The behavioral effects of ethanol which may be anxiolytic, ataxic, and sedative/hypnotic may be exerted by $GABA_A$ receptor-mediated ionic influx enhanced by allosteric mechanism leading to neuronal membrane hyperpolarization [15]. Ethanol binds to and alters the membrane-bound ligand-gated and voltage-dependent ion channels [16].

The ionotropic $GABA_A$ receptor complexes are pentameric composed of distinct subunits, 16 of which have been reported in mammals [17,18]. The genes encoding these receptor proteins are organized in clusters on the chromosomes. GABA gene cluster on chromosome 5 is formed by four genes encoding GABRB2, GABRA6, GABRA1, GABRG2 subunits of the GABAA receptor. Another gene cluster GABRG1, GABRA2, GABRA4 and GABRB1 is present on chromosome 4p12 [19,20]. Several genetic studies, including association and genome-wide association studies, implicate various GABA receptor genes as AD susceptibility candidates [21-30].

Alcohol intake influences liver function as it is majorly involved in metabolizing the alcohol consumed. Previous studies have correlated genetic polymorphisms that affect alcohol metabolism including ALDH, ADH [31] with Gamma Glutamyl Transferase (GGT) levels. Polymorphisms of other neurotransmitter systems like dopamine have also been reported to have an effect on the liver enzymes. A positive effect of the dopamine receptor D1 gene polymorphism (DRD1-48A/G) on Liver Function Tests (LFT) including enzymes Serum Glutamic-Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT), GGT, serum protein, albumin has been reported with the G allele or the GG genotype associated with higher alcohol consumption and impaired LFT in the Indian subjects [32]. Therefore, the present study aimed to explore the role of the Single Nucleotide Polymorphisms (SNPs) in the COMT and GABA pathway genes in subjects of alcohol dependence and also screen for the presence of any correlation with liver function enzymes SGOT/SGPT.

## Materials and Method

The study conformed to the declaration of Helsinki and ethical clearance was obtained from the Institute ethics committee. The present study reports on a total of 100 AD males (110 AD subjects in case of COMT Val158Met) diagnosed on the basis of DSM-IV criteria were recruited from the outpatient department (OPD) of National Drug Dependent Treatment Centre (NDDTC) of the All India Institute of Medical Sciences (AIIMS). 100 healthy males with no history of any substance abuse from the general population formed the control group. The mean age of controls recruited in the study was (31.0 ± 0.49) years while the AD subjects had a mean age of (35.7 ± 0.76) years. The average duration of alcohol use were (13.9 ± 0.73) years with age at first use of alcohol being (21.8 ± 0.58) years and an average alcohol intake (85.1 ± 5.02) g/day (Table 2).

**Inclusion criteria (Cases):** Male aged 18-60 years, diagnosis of AD (as per DSM IV), willing to participate in the study, may or may not be on a treatment to maintain abstinence and current users, i.e., last dose of alcohol within preceding 30 days.

**Inclusion criteria (Controls):** Male aged 18-60 years, no history of any psychoactive substance use disorder (excluding nicotine) in self or in a 1st degree relative.

**Exclusion criteria:** Age<18 years, unwilling to participate, substance use disorder (other than alcohol/ tobacco), evidence of having suffered from major physical/mental illness.

Socio-demographic profile of all the subjects was noted using a semi-structured questionnaire. This questionnaire included information related to the age at first use of alcohol, quantity of alcohol consumed (g/day), duration of alcohol use, and a detailed pedigree upto three generations. The WHO ASSIST v3.0 (Alcohol, Smoking and Substance Involvement Screening Test) questionnaire was also used to evaluate the extent of drug usage. Assist provides specific substance involvement scores that differentiate the subjects into low, moderate and high risk groups. In addition to these, Liver Function Test results SGOT and SGPT were also recorded for each subject.

### Methodology

5 ml peripheral blood drawn from all the individuals was subjected to genomic DNA isolation using the salting out method [33]. It was further processed for PCR amplification followed by restriction digestion to screen for the presence of Val158Met (rs4680) and -287A>G (rs2075507) SNPs of COMT gene and rs13172914 and rs211014 of the GABAA receptor gene. COMT Val158Met, COMT-287A>G and GABRG2 rs211014 were amplified as previously described [34-36]. Primers and restriction enzymes were designed for GABRA6 rs13172914 using Primer3 and NEB cutter software. The primer sequences used for PCR amplification were as follows - Forward primer: 5`-ACGGCTCACAAAATTTGCTT-3`; Reverse primer: 5`-CCACCTCCACCTCAAGTTGT. The restriction enzyme utilized for restriction digestion was HpyCH4V (NEB, USA).

A reaction mixture of 25 µl was prepared using 200 ng genomic DNA, primers (0.5 µM each), $MgCl_2$ (1.5 mM), deoxyribonucleotide triphosphates (dNTPs; 0.2 mM), 1x PCR buffer (Thermo Fisher Scientific,Waltham, MA, USA) and Taq polymerase (0.5 U;Thermo Fisher Scientific,Waltham, MA, USA). PCR amplification was done using ABI 9700 ([ABI], Foster City, CA) followed by restriction digestion (NEB enzymes, USA) and genotyping performed.

### Statistical analysis

Genotype and allele frequency was calculated for all the polymorphisms and difference between cases and controls was assessed by Pearson's chi square test. One way ANOVA was used assess the correlation of genotypes with other parameters like age at first use, alcohol intake/day; Assist score and duration of use using SPSS v21.0 software. PLINK tool was used for calculating minor allele frequencies and to check for Hardy Weinberg equilibrium. PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) is an open-source whole genome association analysis toolset [37]. The software is efficient for analyzing even whole genome data computationally. It can also be used to perform genotype analysis in case-control and family based association studies. All markers were tested for minor allele frequency

(MAF>0.01) and Hardy Weinberg equilibrium at p<0.0001 in both cases and controls.

## Results

The results of the four markers are presented in Table 1 and the demographic details listed in Table 2. All SNPs were in Hardy Weinberg equilibrium and minor allele frequency for the markers was more than 0.01. The genotype frequency of Val/Met genotype of the rs4680 SNP of COMT was significantly higher in AD subjects (0.57) compared to the controls (0.42) (p= 0.03).

| S.no | Genotype frequency | | | | P value | Allele frequency | | P value |
|---|---|---|---|---|---|---|---|---|
| 1 | COMT Val158Met (rs4680) | Val/Val GG | Val/ Met GA | Met/ Met AA | 0.03* | Met | Val | 0.32 |
| | Cases n=110 | 31 (0.28) | 63 (0.57) | 16 (0.15) | | 95 (0.43) | 125 (0.57) | |
| | Controls n=100 | 31 (0.31) | 42 (0.42) | 27 (0.27) | | 96(0.48) | 104 (0.52) | |
| 2 | COMT 287 AG (rs2075507) | A/A | G/A | G/G | 0.4 | A | G | 0.73 |
| | Cases n=100 | 55 (0.55) | 38 (0.38) | 7 (0.07) | | 52(0.26) | 148 (0.74) | |
| | Controls n=100 | 55 (0.55) | 42 (0.42) | 3 (0.03) | | 48 (0.24) | 152 (0.76) | |
| 3 | GABRA6 rs13172914 | C/C | C/T | T/T | 0.4 | C | T | 0.31 |
| | Cases n=100 | 17 (0.17) | 54 (0.54) | 29 (0.29) | | 88 (0.44) | 112 (0.56) | |
| | Controls n=100 | 15 (0.15 ) | 47 (0.47) | 38 (0.38) | | 77 (0.39) | 123 (0.62) | |
| 4 | GABRG2 rs211014 | AA | AC | CC | 0.81 | A | C | 0.58 |
| | Cases n=100 | 10 (0.1) | 42 (0.42) | 48 (0.48) | | 62 (0.31) | 138 (0.69) | |
| | Controls n=100 | 8 (0.08 ) | 40 (0.40) | 52 (0.52) | | 56 (0.28) | 144 (0.72) | |

**Table 1:** Genotype and allele frequency distribution for the COMT (rs4680, rs2075507) and GABAA (rs13172914, rs211014) gene polymorphisms, *p<0.05.

In COMT-287A>G, the genotype and allele frequency did not show significant difference between the cases and controls. Comparison of genotypes with other parameters showed the GG genotype to be significantly associated with raised SGPT levels (p=0.036).

Analysis of the SNPs of GABA pathway did not reveal any difference in the genotype frequency of rs13172914 between cases and controls (p=0.40) although, the major allele T was more pronounced in the controls (0.62) in comparison to the cases (0.56). This marker was also observed to be significantly associated with WHO ASSIST score of severity of AD (p=0.05).Another marker rs211014, did not show any significant difference, nor association with AD.

## Discussion

Alcohol dependence considered to be a multifactorial disorder occurs due to various gene-gene and gene-environment interactions [1,5]. Numerous genetic association studies have been conducted to determine polymorphisms in genes of the dopamine pathway [2,38-51].

Several studies have shown association of Val158 with impulsive behaviour and substance abuse [42,43]. Met158 has been reported to be associated with low activity of enzyme [29,44,45] and literature suggests Asian population in which risk appears to be associated with Met158 [11,29]. Studies by Foroud et al. and Ishiguro et al. did not find any association with AD [10,46].

We observed significant difference between Val/Met genotype between the cases and controls. Patients in our study group showed high frequency of heterozygous genotype which might be responsible for this difference. Our study is in concordance with Vandenberg et al. [43] and Sery et al. [48] in Caucasians utilizing the same methodology (PCR-RFLP), with the high activity allele G (Val158) to be more frequent in the cases. Voisey et al. [47] observed weak association with AD in the Caucasians. Their study however, included a significant proportion of patients who presented with two or more severe medical conditions including pancreatitis, cirrhosis, hepatitis or peripheral neuropathy. Contrasting results were observed by Ishiguro et al. [10] who also utilized PCR-RFLP but reported no difference in allele frequency distribution between 175 cases and 354 age and sex matched Japanese subjects. A family based association study by Foroud et al. [46] indicated that presence of COMT (Val158Met) polymorphism did not affect the pathology of alcohol consumption and smoking in European-Americans. Thus, irrespective of the differences in the methodology, the contradictory findings are likely due to differences in ethnicity, type of study (case-control/ family based association study) demographic differences, etc.

| S.No | Variables | Cases (N=100) (Mean ± SE) | Controls (N=100) (Mean ± SE) |
|---|---|---|---|
| 1 | Age (Years) | 35.7 ± 0.76 | 32.0 ± 0.48 |
| 2 | Age at first use (Years) | 21.8 ± 0.58 | - |
| 3 | Duration of Alcohol use (Years) | 13.8 ± 0.73 | - |
| 4 | Alcohol intake (g/day) | 85.1 ± 5.02 | - |
| 5 | WHO Assist score for Alcohol (Assist 1b) | 28.7 ± 0.58 | - |
| 6 | SGOT | 71.5 ± 5.10 | 37.2 ± 1.65 |
| 7 | SGPT | 64.3 ± 5.88 | 43.5 ± 2.29 |

**Table 2:** Demographic details of the cases and controls enrolled in the study.

Another COMT gene SNP rs2075507 in the promoter region was observed to be associated with heroin dependence [12]. Later, this SNP was observed to be associated with obsessive-compulsive disorder in Chinese Han population [49]. In our study we observed the frequency of both alleles of rs2075507 to be similar in cases and controls. On correlation with liver function enzymes' status, this polymorphism was found to be significantly associated with raised SGPT levels (p=0.036). Previous study by Suhartini et al. [31] explains that presence of polymorphism in the genes of Aldehyde dehydrogenase might affect the function of liver as suggested by raised gamma glutamyl transferase (GGT) levels in the subjects having the variant genotype. Gene polymorphisms may be associated with certain disorders due to their direct/indirect effect on the rate of transcription and levels of the proteins synthesized as seen in liver dysfunction due to presence of certain SNP's. We observed raised SGPT levels in subjects with GG genotype (103.75 ± 31.8) as compared to those with AA genotype (76.23 ± 11.1). A significant association with SGPT as observed in our study suggests a possibility of liver dysfunction in cases with genotype GG, although role of this polymorphism has to be studied further for assessing its role in liver dysfunction.

The involvement of $GABA_A$ receptor is implicated in a number of complex disorders, including substance abuse. Among the commonly studied $GABA_A$ receptor gene variants associated with substance dependence none have been determined to be functional or pathogenic. A meta-analysis of the variants in the GABAA receptor genes (GABRB2, GABRA6, GABRA1, and GABRG2 on chromosome 5q and GABRA2 on chromosome 4p12) by Li et al. showed GABRG2 rs211014 to be associated with both alcohol and heroin dependence [28]. Previously, Radel et al. identified sib-pair linkage of the 5q34 GABAA receptor genes to alcohol dependence in Finns [50]. Another association study on the Chinese male subjects by Loh et al. reported the rs211014 to be significantly associated with heroin dependence [36]. In contrast, our population did not show any change in the frequencies with respect to this marker showing homogeneity of the population.

Li et al. [28] reported positive association with GABRG2 rs211014 in a combined meta-analysis on alcohol, opioid and methamphetamine dependence. The discrepancy in results from other association studies is likely due to differences in ethnicity of the population studied; therefore some SNPs important for a particular population may not be a susceptibility factor for another population. Additionally, diagnostic classification used for recruiting subjects may vary (DSM IV/ International Classification of Diseases (ICD-10)) in different association studies. According to Pritchard et al. [51] population stratification is another crucial factor in case-control studies resulting in spurious associations. This is possible when the patients and controls drawn from a population pool differ in allele frequency due to different ancestry. Therefore, null findings could be a possibility in any association study.

Variation in GABRA6 has been observed to be associated with increased production of cortisol and increased blood pressure in response to psychological stress [52]. A few studies have also reported its role in anxiety and depression [53]. The GABRA6 variant rs13172914 is a less studied SNP with only one report by Pham et al. indicating a negative association of the commonly studied GABA receptor genes with anxiety spectrum disorder [54]. The same SNP studied in our subjects also did not show any association with AD, suggesting its negative role. The polymorphism, albeit was observed to be significantly associated with WHO assist score, a severity index for alcohol dependence.

## Conclusion

The present study attempted to identify association, if any of COMT and GABA pathway gene SNPs with alcohol dependence. The findings suggest a possible association of COMT Val158Met (rs4680) with AD in our patients, while the COMT rs2075507 was observed to be associated with liver enzyme SGPT suggesting a possibility of the role played by this variant in liver dysfunction, while the GABA pathway polymorphism GABRA6 rs13172914 was seen to be associated with severity of alcohol dependence. These are preliminary results from subjects covering a large part of North India. Future studies on larger data sets may substantiate the role of these and other polymorphisms in alcohol dependence.

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