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Original Research

Prevalence of COMT Val158Met polymorphism in Eastern UP population

P. Kumar, U. Yadav, V. Rai*

Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur-222003, Uttar Pradesh, India

Correspondence to: raivandana@rediffmail.com

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Abstract: Catechol-O-methyltransferase (COMT) is an abundant S-adenosylmethionine (SAM-)-dependent methyltransferase that methylates catechol compounds, including catecholamines and catecholestrogens.COMT gene located at chromosome 22q11.2 contains a functional polymorphism at codon 158(Val-158Met), which has been related to psychiatric diseases and different types of cancer. COMT might affect tHcy levels because as a by-product it converts SAM to S-adenosylhomocysteine (SAH), which is reversibly converted to homocysteine. The aim of the present study was to determine the frequency of COMT Val158Met polymorphism in scheduled caste (SC) population of Jaunpur district. Total 100 healthy unrelated subjects belonging to SC, between the age group of 18 to70 years were randomly selected for the present study. 3 ml blood samples were collected from each subject. The inclusion criteria of subjects for present study are that they should be domicile of Uttar Pradesh, and healthy without any individual/ family history of genetic or metabolic disorders. COMT Val158Met polymorphism analysis was done by PCR-RFLP method. The Val/Val genotype was found in 48 subjects, Val/Met in 40 subjects and Met/Met genotype in 12 subjects. Genotype frequencies of Val/Val, Val/Met and Met/Met were 0.48, 0.40 and 0.12 respectively. The allele frequency of Val allele was found to be 0.68 and Met allele frequency was 0.32.

Key words: Catechol-O-methyltransferase; COMT; Val158 Met; Genotype; Allele; Eastern UP.

Introduction

Catechol-O-methyltransferase (COMT) is an intracellular methylation enzyme that catalyzes the first step of the dopamine degradation pathway and inactivates catecholamines, which include dopamine, epinephrine, and norepinephrine. It is accepted widely that COMT plays a crucial role in modulating nerve function and physiology, due to its broad distribution throughout the brain and various peripheral tissues. COMT enzyme occurs in two distinct isoforms: a smaller soluble protein in the cytoplasm (S-COMT; 221 aa) and a longer membrane-bound isoform (MB-COMT 271 aa) (1). The MB-COMT is predominantly expressed in brain neurons, while the S-COMT is predominantly expressed in blood cells and tissues like liver and kidney (1).

The COMT gene is located on chromosome 22q11.1–q11.2 and contains six exons. A single base pair change (G471 A) in exon 4 of the COMT gene, at position 472 in the long mRNA, and 322 in the short mRNA, results in an amino acid change (Val--> Met), at codon 158 of MB-COMT and codon 108 of S-COMT, which decreases the activity level of the COMT enzyme 3 to 4 fold (2,3). In addition to being a functional polymorphism, this SNP also creates a polymorphic NlaIII restriction site in the DNA. The two alleles are referred to as Val or COMT*H, or the NlaIII site-absent (G; Val) allele that encodes the thermostable, high activity enzyme and Met or COMT*L, or the NlaIII site-present (A; Met) allele that encodes the thermolabile, low activity enzyme (3,4,5).Presence of a methionine at position 158 decreases the thermostability of COMT and reduces the activity of the enzyme to 25% of that of the COMT 158-Val enzyme, which leads to diverse changes in cognitive function and human physiology (6).Both the alleles are co-dominant, individuals having Val/Met genotype have an intermediate level of COMT activity in comparison to homozygous (Val/Val) individuals (3).Very limited data about COMT Val158Met mutation frequency are available from Indian population, and no data are available about Uttar Pradesh population; hence, the aim of the present study is to estimate frequency of COMTVal158Met polymorphism in healthy individuals of Eastern Uttar Pradesh.

Materials and Methods

3 ml blood samples were collected from randomly selected 100 unrelated healthy individuals belonging to scheduled caste (SC) population of Eastern Uttar Pradesh belonging to both the genders (50 males and 50 females). All subjects were between the age group of 18–70 years. All subjects gave their informed written consent and the study was approved by the Institutional Ethics Committee of VBS Purvanchal University, Jaunpur. A questionnaire was used to collect demographic information, personal medical history and family history. The inclusion criteria of subjects were- (i) subjects should be domicile of Eastern Uttar Pradesh, (ii) subjects should be belong to scheduled caste, (ii) subjects should be healthy, without any individual/ family history of genetic disorder and (iv) subjects should be unrelated and randomly selected from the Eastern UP population.

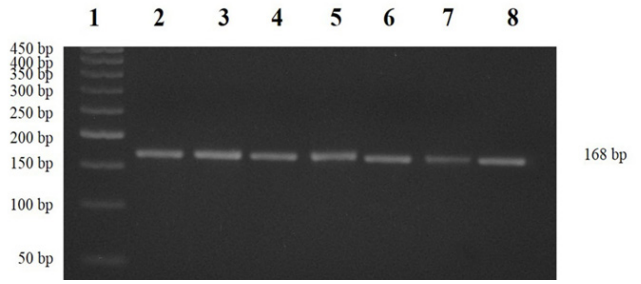

Figure 1. Agarose gel picture showing 168 bp long COMT amplicon.

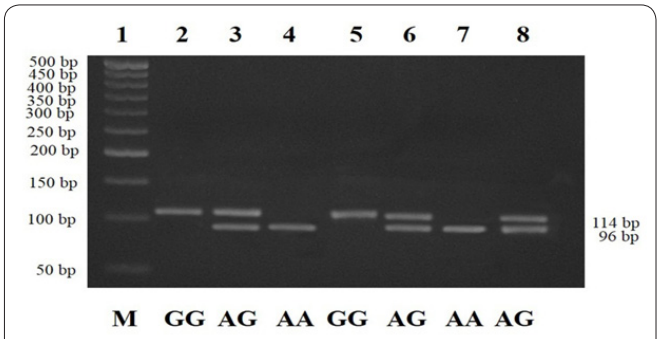

Figure 2. Agarose gel picture showing NlaIII digested amplicon of COMT gene showing different three COMT genotypes.

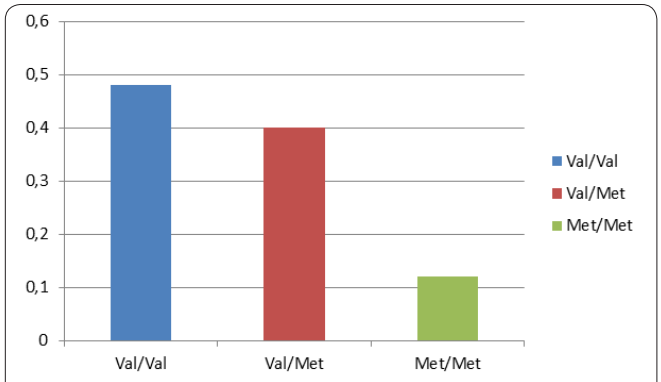

Figure 3. Bar diagram showing frequency of three COMT genotypes-homozygous wild, heterozygous and mutant homozygous in Eastern UP population.

Genomic DNA was extracted according to the method of Bartlett and White (7) with slight modification. Genomic DNA was amplified for COMT Val108/158Met polymorphism analysis by polymerase chain reaction (PCR) by previously described method of Kahayan et al (8), using primer sequences F5′-CTGTGGCTACTCAGCTGTG-3′ and R5′-CCTTTTTCCAGGTCTGACAA-3′. The amplified product was digested with NlaIII restriction enzyme (Genei, India) to identify the COMT allele. Amplification and restriction products were analyzed by electrophoresis in 2% and 4% agarose (Fermentas) gels, respectively.

Results

Figure 1 showed agarose gel, illustrating 168bp long amplified fragment of COMT gene. After NlaIII restriction digestion of amplicon, the wild Val/Val genotype produced three bands of 114,29 and 25 bp long fragments. The heterozygote Val/Met produced five bands of 114, 96, 29, 25 and 18bp long fragments. Mutant Met/Met homozygote produced four fragments of 96, 29,25 and 18bp. Fragments of 29,25 and 18 bpsize were not visualized in 4% agarose gel. Hence, presence of single 114bp long band indicated homozygous wild genotype (Val/Val), presence of single 96bp long band indicated mutant homozygous genotype (Met/Met) and presence of both 114 and 96 bp long fragments indicated heterozygous genotype (Val/Met). Figure 2 shows an agarose gel illustrating different genotypes of the Val158Met polymorphism. Allele frequencies and genotype distributions observed in the present study are presented in Table 1. The prevalence of Val/Val, Val/Met, and Met/Met genotypes determined in the target population were 48, 40 and 12 respectively (Figure 3). The genotype frequencies of Val/Val, Val/Met, and Met/Met were 0.48, 0.40, and 0.12, respectively. The frequencies of wild (Val) and mutant (Met) alleles were 0.68 and 0.32 respectively. This polymorphism was compatible with Hardy-Weinberg equilibrium (x^2= 0.65; df=2; P=0.41).

Discussion

The Val158Met polymorphism of the COMT gene is functional, easily detectable, and significantly related to metabolism of catecholamines, which underlie pathogenesis of a significant number of mental disorders.

COMT Val158Met polymorphism was investigated in several populations and countries like- Australia (9), Brazil (10), Canada (11), China (12), France (13), Finland(11), Germany (14), Hungary (15), Iran (16), Japan (17), Mexico (18), Norway (19), Poland (20), Slovenia (21), Spain (22), Syria (23), Thailand (24), Turkey (25), UK (26),and USA (27).The frequency of the mutant Met allele vary greatly among the populations studied, frequency of Met allele is reported 0.56 in American (28),0.5 in European (29),0.27 in Chinese(30,31), 0.31 in Han Chinese (32),0.35 in Japanese (33),0.64 in European Hispanic (34),0.38 in American Hispanic(35),and 0.43 in Spanish (36) populations. Population frequency of this clinically important polymorphism is not well reported from Indian population, only few reports are available which are based on the case-control studies (37,38).Frequency of Val158Met polymorphism observed in the present study is well comparable with the frequency reported in earlier studies published from Indian and Asian populations.

This common and functional Val108/158Met COMT polymorphism has been investigated in relationship with many psychiatric disorders, like schizophrenia (30**),** bipolar disorder (32), unipolar disorder (33), attention-deficit hyperactivity disorder (39), anorexia nervosa (40),

Table 1. COMT genotype and allele frequency distribution among Eastern Uttar Pradesh population.

	Genotype			Allele	
	Val/Val	**Val/Met**	**Met/Met**	**Val**	**Met**
Number	48	40	12	136	64
Frequency	0.48	0.40	0.12	0.68	0.32

autism (41), suicide (42), and drug abuse (43). In these studies, an association, and also a lack of association, between the COMT Val108/158Met polymorphism and the studied psychiatric diseases/ disorders were reported.

In conclusion, the finding of present study is that COMT Met allele frequency in healthy individuals of Eastern Uttar Pradesh is 32%. The results of our study on COMT Val158Met polymorphism in the SC population supplement the variability of this gene worldwide and can serve as a basis for further associative investigations on the role of COMT in susceptibility to different psychiatric disorders in the populations of different ethnic descent. Screening of populations for this clinically important gene polymorphism is also needed for proper counseling strategies.

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