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Uttar Pradesh Methionine Synthase Reductase A66g Polymorphism in Rural Population of Uttar Pradesh (India)

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Abstract: The present study was aimed to analyze methionine synthase reductase (MTRR) A66G polymorphism in Uttar Pradesh (UP) population and PCR-RFLP method was used for the mutation analysis. Total 104 samples were analyzed and AA genotype was found in 10 individuals, AG genotype in 67 individuals and GG in 27 individuals. The frequencies of AA, AG and GG genotypes in the present study were also assessed with the expected distribution (i.e. Hardy-Weinberg equilibrium) by using χ^2 test. The genotype frequencies of AA, AG and GG were 0.096, 0.644 and 0.259, respectively. Allelic frequencies of A and G were 0.418 and 0.581. It was reflected from the results of the present study that the percentage of heterozygous genotype (AG) is highest in the target population.

Key words: Methionine synthase reductase, MTRR, homocystinuria, genotype, allele, A66G polymorphism

INTRODUCTION

Methionine synthase reductase (MTRR) gene is located on chromosome 5p15.2-15.3 and enzyme catalyzes the conversion of the inactive form of methionine synthase (MTR) into its active form, by regeneration of methyl (III) cobalamin, the cofactor of MTR. Mutation analysis of homocystinuria patients with severe deficiency of methionine synthase reductase led to the discovery of a mutation that is common in the general population (Wilson *et al*., 1999). Wilson *et al*. (1999) identified this common variant of MTRR (A66G) in which methionine replaces isoleucine in the enzyme with a reported population frequency of ~30% (Wilson *et al*., 1999; Gaughan *et al*., 2001; Rady *et al*., 2002; Brilakis *et al*., 2003). The I22M variant is located in the putative FMN-binding domain of the MTRR enzyme that is suggested to interact with methionine synthase (Leclerc *et al*., 1998). MTRR A66G polymorphism was associated with an increase in plasma homocysteine, with the GG genotype having a greater effect than the AG genotype (Gaughan *et al*., 2001; Rady *et al*., 2002). During homocysteine/methionine cycle, methionine is synthesized from homocysteine by Vitamin B_{12}-dependent methionine synthase. The methyl groups released upon the transformation of methionine to homocysteine (S-adenosylmethionine being the most important methyl-group donor) facilitate the methylation of DNA, lipids and proteins. Higher level of homocysteine is itself toxic and an independent risk factor for several complex disorders like-cardiovascular disease (Robinson *et al*., 1995), Alzheimers disease (McCaddon *et al*., 1998), Schizophrenia (Applebaum *et al*., 2004) end stage renal disease (Van Guldener and Stehouwer, 2003), type II diabetes (De Luis *et al*., 2005) and Neural tube defects (Mills *et al*., 1995) etc. After Methylenetetrahydrofolate reductase (MTHFR), Methionine synthase reductase (MTRR) is the second enzyme providing an active state for the remethylation of homocysteine to methionine and when MTRR enzyme variants is coupled with MTHFR polymorphism the combined deleterious effect may be additive. Several reports about MTHFR C677T mutation frequency are available for Indian population (Bhat *et al*., 2008; Saraswathy *et al*., 2008) but not a single report regarding frequency of MTRR A66G polymorphism is available from India and the rural population of Uttar Pradesh is genetically unexplored. Hence, the present study aimed to evaluate the mutant allele (G) frequency in the rural area of Uttar Pradesh.

MATERIALS AND METHODS

Samples: Total 104 healthy individuals were included in the present study which are unrelated and randomly

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selected from the rural area of the UP. Out of which 70 were males and 34 were females. Three milliliter blood was collected from each subject by veinipuncture in EDTA coated vials. Informed consent was obtained from each subject. The present study was conducted in the Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur, India during the period 2008-2009.

Genomic DNA extraction: Genomic DNA was extracted according to the method of Bartlett and White (2003) and extracted genomic DNA was kept at -20°C until the genotype analysis.

Genotype analysis: PCR-RFLP method was used for the genotype analysis. For amplification primers as described by Wilson *et al.* (1999) were used. The primers 5-'GCAAAGGCCATCGCAGAAGACAT-3' and 5'-GTGAAGATCTGCAGAAAATCCATGTA-3' were used to amplify 66-bp fragment. PCR was performed in Multi Gene II thermo cycler (Labnet, USA) and the profile consisted of an initial melting step of 4 min at 94°C, followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 65°C, 1.30 min extension at 72°C and a final elongation step of 10 min at 72°C. Amplification and restricted products were analyzed by electrophoresis in 2 and 4% agarose (Fermentas) gels, respectively. Allele frequencies were calculated using the gene counting method. χ^2 test was performed to test Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Amplification with MTRR gene specific primer generated 66 bp amplicon (Fig. 1) and after Nde I digestion homozygous AA genotype produced two bands of 44 and 22 bp, heterozygous AG genotype produced three bands 66,44 and 22 bp and GG genotype remained uncut (Fig. 2).

The distribution of the MTRR genotypes (AA, AG and GG) within the study population is shown in Table 1, total 104 samples were analyzed for the present study. Out of which AA genotype were found in 10 (9.61%) individuals, AG genotype in 67 (64.42%) individuals and GG in 27 (25.96%) individuals (Fig. 3). The frequencies of AA, AG and GG genotypes in the present study were also assessed with the expected distribution (i.e., Hardy-Weinberg equilibrium) by using χ^2 test. The genotype frequencies of AA, AG and GG were 0.096, 0.644 and 0.259, respectively. Allelic frequencies of A and G were 0.418 and 0.581. It was reflected from the results of the present study that the percentage of heterozygous genotype (AG) is highest in the target population.

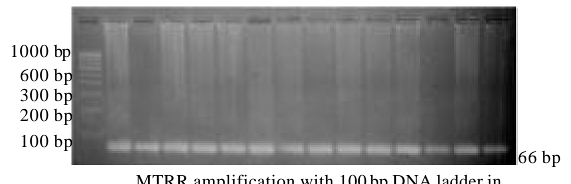

Fig. 1: MTRR amplification product of 66-bp for A66G polymorphism

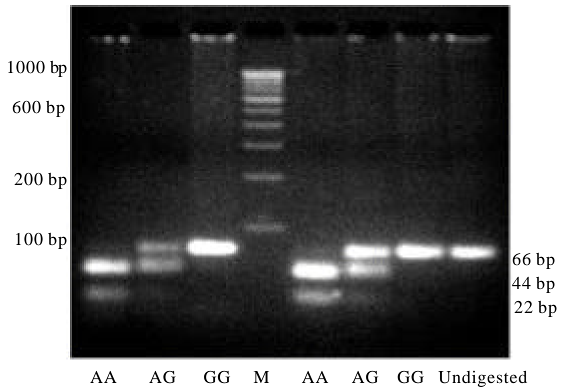

Fig. 2: Nde I digested A66G polymorphism analysis. Lane 1 and 5: AA (normal homozygous); Lane 2 and 6: AG (hetrozygous), Lane 3 and 7: GG (mutant homozygous), Lane 4: M (Marker) 100-bp ladder; Lane 8: Undigested amplicon

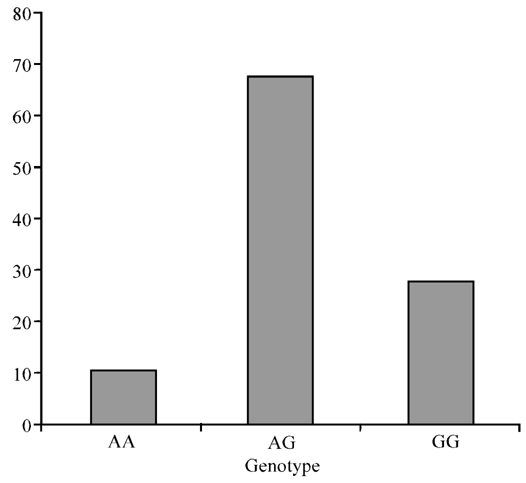

Fig. 3: Bar diagram showing AA, AG and GG genotypes in UP population

Table 1: MTRR genotype and allele frequency distribution among Uttar Pradesh population

	Genotypes			Alleles	
	AA	AG	GG	A	G
Number	10.00	67.00	27.00	87.00	121.00
Percentage	9.61	64.42	25.96	41.82	58.17
Frequency	0.096	0.644	0.259	0.418	0.581

The world-wide frequency of A66G polymorphism is ~30% (Wilson *et al*., 1999; Gaughan *et al*., 2001; Brilakis *et al*., 2003). However, its frequency varies in different ethnic and geographical regions as reported by Rady *et al*. (2002) the lowest frequency in the Hispanic population (28.65%) compared to 34% among African-Americans, 43.1% among Ashkenazi Jews and 54.45 among Caucasians (54.4%). In present study the frequency of G allele (0.581) is also higher than the A allele and is comparable with the Caucasian population (Table 1).

Olteanu *et al*. (2002) have reported that the I22M variant (A66G) MTRR enzyme exhibits four-fold lower activity than the wild-type protein in the reactivation of MTR *in vivo*. Hence the level of active MTR is reduced and so the availability of SAM, as methyl donor is also decreased, thus leading to DNA hypomethylation and it was pointed out by several studies that the DNA hypomethylation is the main causative factor in the chromosome missegregation, micronucleus formation and defective gene expression etc (Zijno *et al*., 2003). Missegregation of chromosomes and altered gene expression are the main causative factors behind the role of A66G polymorphism and as risk factor of hereditary disorders (Hobbs *et al*., 2000; Lee *et al*., 2006). Several epidemiological and case control studies have already reported that the GG genotype may be a risk factor for several disease/disorders like Neural tube defects (Bailey *et al*., 2001; Gos and Szpecht-Potocka, 2002; Pietrzyk *et al*., 2003; Relton *et al*., 2004; Van der Linden *et al*., 2006), Down syndrome (Hobbs *et al*., 2000; Scala *et al*., 2006; Coppede, 2010), Coronory artery disease (Brilakis *et al*., 2003), male infertility (Lee *et al*., 2006; Ravel *et al*., 2009), Cancer (Zhang *et al*., 2007) etc. Screening of different Indian population for such clinically important gene polymorphism is urgently needed for proper genetic counselling and disease management strategies.

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