Original Article

Prevalence of methylenetetrahydrofolate reductase C677T polymorphism in eastern Uttar Pradesh

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AIM: This study was aimed to evaluate the 5, 10-methylenetetrahydrofolate reductase (MTHFR) C677T mutation in eastern Uttar Pradesh population.
MATERIALS AND METHODS: Polymerase chain reaction (PCR) using specific primers followed by amplicon digestion by Hinf I restriction enzyme was used for MTHFR C677T polymorphism analysis. Total 250 subjects were analyzed.
RESULTS: The CC genotype was found in 192 subjects, followed by CT in 56 subjects and TT in 2 subject. Genotype frequencies of CC, CT and TT were 0.768, 0.224 and 0.008, respectively. The frequency of C allele was found to be 0.88 and that of T allele was 0.12.
CONCLUSION: It is evident from the results of the present study that the percentage of homozygous genotype (CC) is highest in the target population.

Key words: Allele, C677T polymorphism, genotype, homocysteine, methylation, methylenetetrahydrofolate reductase, Methylenetetrahydrofolate reductase

Introduction

Methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate-dependent homocysteine metabolism, and severe enzyme deficiency results in elevated plasma homocysteine concentration and ultimately in syndrome homocystinuria characterized by multiple physical, developmental, and cognitive defects. Elevated serum or plasma homocysteine (tHcy) is a risk factor for a series of pathologic conditions, including cardiovascular disease,[1] Alzheimer disease and cognitive dysfunction,[2] type II diabetes,[3] and neural tube defects,[4] etc. MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate reductase, which donates methyl group for the conversion of homocysteine to methionine. The methyl cycle supplies 1-carbon units critical for a variety of methylation reactions essential for proper gene expression and maternal and paternal imprinting by methylated DNA.

The human MTHFR gene is 20 kb long (20,336 bp) and mapped at 1p36.3 (OMIM 607093), having 11 exons. More than 40 polymorphisms have been described in MTHFR, but the most common and clinically important variants are C677T in exon 4[5] and A1298C in exon 7.[6] The C677T variant results from a single nucleotide substitution mutation at 677th position of the gene, in which cytosine is replaced by thymine. At protein level this change makes substitution of alanine to valine at 222nd amino acid. This change makes enzyme thermolabile with reduced enzymatic activity. This deficiency is inherited as an autosomal recessive trait. Individuals who are homozygous for the thermolabile variant of MTHFR (TT) have an increased risk of hyperhomocysteinemia and lower levels of folate in plasma and red blood cells.[7] C677T mutation has been reported to be a genetic factor for several disease such as neural tube defects,[4,8,9] cardiovascular diseases,[5,10] and psychiatry disorders.[11-13] Very limited data about MTHFR C677T mutation frequency are available for Indian population,[14-18]

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and no data are available on Uttar Pradesh population; hence, the aim of the present study is to estimate frequency of C677T polymorphism variant of the MTHFR gene in UP population.

Materials and Methods

Samples

Total 250 subjects between the age group of 18–70 years were randomly selected for the present study from the rural area of Jaunpur district of Uttar Pradesh. Out of which 168 were males and 82 were females. 3 ml blood samples for genetic analysis were collected in Ethylene diamine tetraacetic acid disodium salt (EDTA) -coated vials and informed consent was obtained from each subject. Only unrelated individuals participated in the study. The present study was conducted in the Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur, India, during the period 2008–2010.

Genomic DNA extraction

Genomic DNA was extracted according to the method of Bartlett and White[19] with slight modification. Extracted DNA was stored at - 20°C until the genotype analysis was performed.

MTHFR genotype determination

Analysis of the MTHFR C677T mutation was based on the method of Frosst *et al.*[5] Polymerase chain reaction (PCR) was performed using genomic DNA and the primers 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'and 5'-AGGACGGTGCGGTGAGAGTG-3' to generate a 198-bp fragment. PCR was performed in MJ Mini thermo cycler (Bio-Rad, USA), and the program consisted of an initial melting step of 2 min at 94°C, followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 65°C, 1 min extension at 72°C, and a final elongation step of 10 min at 72°C. The amplified product was digested with Hinf I restriction enzyme (Genei, India), which cleaves only mutant MTHFR allele into 175- and 23-bp fragments. Amplification and restriction products were analyzed by use of electrophoresis in 2% and 4% agarose (Fermentas) gels, respectively.

Results and Discussion

Amplification with MTHFR specific primers produced 198 bp long amplicon [Figure 1]. After digestion with Hinf I wild homozygous (CC) remained uncut, and produced one band of 198 bp, homozygous mutant produced two bands (175bp and 23bp) and heterozygous (CT) genotype produced three bands (198bp, 175bp and 23 bp) in agarose gel electrophoresis [Figure 2]. Genotype distribution and allele frequencies in the present study are presented in Table 1. The CC genotype

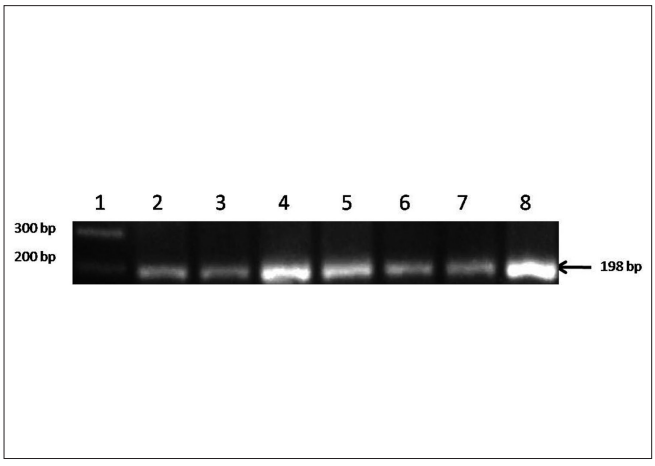

Figure 1: Amplified product of 198-bp for C677T polymorphism

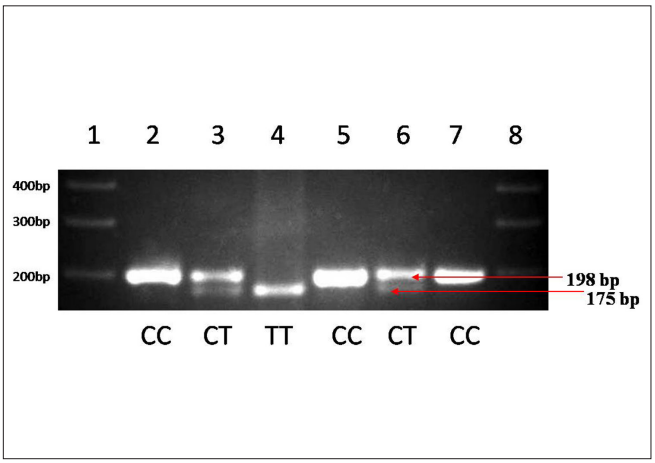

Figure 2: RFLP analysis for the C677T mutation on 198-bp MTHFR PCR products with Hinf I. Wild-type homozygous remains uncut after Hinf I digestion gives one band, mutant homozygous gives two bands (175-bp and 23-bp), and heterozygous gives three bands(198-bp,175-bp, and 23-bp).The figure shows normal CC (lanes: 2, 5, and 7), heterozygous CT (lanes: 3 and 6), and homozygous mutant TT (lane: 4) genotypes

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

	Genotype			Alleles	
	CC	CT	TT	C	T
Observed Number	192	56	02	440	60
Frequency	0.768	0.224	0.008	0.88	0.12

was found in 192 subjects (76.8%), followed by CT in 56 subjects (22.4%) and TT in 2subject (0.81%). The conformity of the genotype frequency distribution to Hardy–Weinberg proportion was examined using X2 test. Genotype frequencies of CC, CT and TT were 0.768, 0.224 and 0.008, respectively. The frequency of C allele was found to be 0.88 and that of T allele was 0.12. It is evident from the results of the present study that the percentage of homozygous genotype (CC) is highest in the target population.

Ample data on the worldwide frequency of this variant are currently available.[14,17,20-26] The frequency of the MTHFR 677T allele varies substantially in different regions of the world and among ethnic groups. For example, the allele frequency ranges from 0.20 to 0.55 in Europeans and from 0.04 to 0.38 in Asian populations.[9,27,28] The T allele frequency (0.12) in our target population is fell within the range of T allele frequency (0.04 to 0.38) reported in other studies published about the Asian populations. The result of our study on MTHFR polymorphism supplements the variability of this gene worldwide and can serve as a basis for further association studies on the role of MTHFR mutation in the susceptibility of different multifactorial diseases.

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