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GENOTYPE PREVALENCE AND ALLELE FREQUENCIES OF 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T MUTATION IN TWO CASTE GROUPS OF INDIA

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Abstract

The aim of the present study was to investigate the distribution of 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphism in two caste group populations of eastern Uttar Pradesh. This mutation has been suggested to be positively associated with the risk of several congenital and multifactorial disorders. Frequency of mutant T allele differs in various ethnic and geographical populations of the world. MTHFR C677T mutation analysis was carried out by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method and the samples studied were randomly selected from the healthy individuals belonging to two caste populations. In Brahmin samples, genotype frequencies of CC, CT and TT were 0.727, 0.25 and 0.023 respectively whereas in Rajput samples, CC genotype was observed in 88 samples, CT genotype in 25 and TT genotype was found in 2 samples. Frequency of mutant T allele was found to be 0.147 in Brahmin and 0.126 in Rajput populations. The percentage of CT genotype and C allele were high in both the populations.

Key words: Genotype, MTHFR, C677T, Polymorphism, PCR-RFLP, Methylation, Folic acid.

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INTRODUCTION

Folate is an important nutritional factor and its principal biochemical role in humans is the transfer of single carbon molecules for various biological reactions (74). Folate plays an integral role in DNA synthesis, DNA stability, DNA repair and DNA methylation; as an epigenetic regulator of gene expression (29,74). Folate deficiency due to low dietary intake or impaired absorption or metabolism may result in increased number of DNA strand breaks, impaired DNA repair, enhanced mutagenesis and alterations in DNA methylation pattern (14,30). The methylenetetrahydrofolate reductase (MTHFR (EC 1.5.1.20)) is the most critical enzyme which plays a central role in the metabolism of folate and homocysteine. This enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5, methyltetrahydrofolate, the predominantly circulating form of folate and the methyl donor for remethylation of homocystein to methionine. Methionine is precursor for the synthesis of S-adenosylmethionine (SAM), the major cellular methyl donor for DNA, RNA, protein and phospholipid methylation. The human MTHFR protein has two isoforms with molecular weight 77kDa and 70kDa. The smaller one was discovered only in fetal and adult liver tissues and kidney. MTHFR has two domains; catalytic one (N-terminal end, 40 kDa) binding FAD, NADPH and methylenetetrahydrofolate, and regulatory domain (C- terminal end, 37kDa) (21). MTHFR gene (MIM236250) has been localized in chromosome 1(1p36.3) and has 11 exons. Its promoter region has several transcription factor binding sites, but does not have TATA-box sequence. It shares similarity with promoter sequences of other genes for example cystathione synthase, methionine synthase and methionine synthase reductase (21). Several SNPs are reported in MTHFR gene but C677T mutation (rs 1801133) is the most common and clinically important (18), in which a cytosine is replaced by thymine at 677th position of the gene (exon 4), converts an alanine to valine at position 222 in protein. C677T mutation was shown to render the enzyme thermolabile and homozygote variants (TT) have 30 percent enzyme activity in comparison with homozygous for wild-type C allele, while heterozygote's (CT) retain 60 percent of wild-type MTHFR activity. The C677T mutation changes the secondary structure of the peptide and interactions between monomers. The variant protein loses its cofactor FAD more quickly and has lower stability. The mutation effect can be suppressed by addition of folate, which causes a higher FAD affinity and an increase in MTHFR stability (21). Individuals with the MTHFR C677T genotype are considered to have increased dietary requirements because they have lower red cell folate levels compared with those without this variant (7) and are found to have higher homocysteine levels only where folate is below the median within a TT genotype population (8). C677T polymorphism has been reported as a risk factor for several diseases and /or clinical conditions including Down syndrome (11,25,75), Neural tube defects (19,73), orofacial clefts (10,37), type I diabetes [8], cardiovascular diseases (18,35), male infertility (57), Schizophrenia (28,65), bipolar disorder (28) and cancer (32,77) etc.

In the present study, the subjects were selected from a heterogeneous population which is supposed to have originating from a common so called Ancestral North Indian Gene pool (58). However, Indians mating pattern and other

cultural and religious practices do make an impact on the population structure. In the present Indian society especially in rural area a caste system is prevalent and caste is defined as collection of people who share similar cultural and religious values and practices. Members within a caste generally marry among themselves (endogamous), intercaste marriages are cultural taboo. These social regulations governing the institution of marriage have resulted in a sub structuring of the Indian gene pool. There are also elaborate social regulations of avoidances of marriages within castes and thus there is genomic subtraction even within gene pool. Impact of deep seated caste system and practice of strict endogamy/marriage rule potentially make every caste population a unique, isolated and important group. Various cast groups like Brhamin, Rajput (Kshatriya), backward caste and scheduled caste are included in the Hindu group. It has been well established that the frequency of C677T variant vary greatly between the populations of different countries. Hence, the present study was designed to determine the frequency of this clinically important variant in two caste groups (Brahmin and Rajput) domicile to Uttar Pradesh, India. Further we compared the allele frequencies (C and T) for the Uttar Pradesh population as a whole with earlier reported frequencies for different regions of India.

MATERIALS AND METHODS

Blood samples from 203 healthy unrelated subjects were collected, out of which 88 subjects belong to Brahmin caste and 115 subjects belong to Rajput caste. Ethical clearance certificate was taken from the Institutional Ethics Committee of VBS Purvanchal University Jaunpur. Written informed consent along with some profile details was taken from each subject prior to blood sample collection. Polymorphism analysis was conducted in the Department of Biotechnology, VBS Purvachal University, Jaunpur, India during the period 2008-2009. The study exclusion criteria were as follows; age below 18 and above 65, family history of genetic and psychiatric disorders, presence of any malignancies and/or metabolic disorders. Genomic DNA was extracted according to the method of Bartlett and White (7). MTHFR C677T polymorphism was identified by PCR amplification followed by digestion with restriction enzyme HinfI. The primers, PCR conditions and HinfI digestion conditions were as previously described by Frosst et al. (18). PCR was performed in MJ Mini thermal cycler (Bio-Rad, USA). Restriction enzyme digested products were analyzed in 4% agarose (Fermentas) gel electrophoresis. Allele frequencies were calculated using the gene count method and x^2 test was performed to test Hardy –Weinberg equilibrium with respect to each population. All statistical analysis was done by statistical software WinPepi (version9.9).

RESULTS AND DISCUSSION

Genotype number and allele frequencies observed in two populations were given in the table 1 and 2. Amplification with MTHFR gene specific primer generated 198-bp amplicon (Figure 1), and after Hinf I digestion homozygous TT genotype produced two bands of 175-bp and 23-bp, heterozygous CT genotype produced three bands 198-bp, 175-bp and 23-bp and CC genotype remained uncut (Figure 2). In Brahmin case, CC genotype was found in 64 subjects, followed by CT genotype in 22 subjects and TT genotype in 2 subjects. Genotype frequencies of CC, CT and TT were 0.727, 0.25 and 0.023 respectively. The Allele frequency of mutant T allele was 0.147 and C allele was 0.852 (x^2= 0.004; p=0.997) (Table 1). In Rajput samples, CC, CT and TT genotypes were found in 88, 25 and 2 subjects respectively. The genotype frequencies of CC, CT and TT were 0.765, 0.217 and 0.017 respectively. Frequency of T allele was 0.126 and frequency of C allele was found to be 0.873 (Table 2). The percentage of CT genotype was higher in both the populations and similarly the frequency of C allele was also high in both the populations.

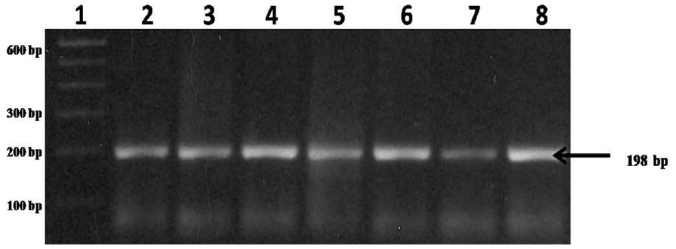

Figure 1. Agarose gel picture showing 198 bp amplicon of exon 4 of MTHFR

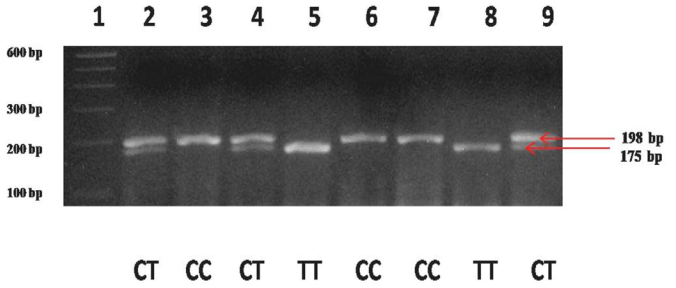

Figure 2. Agarose gel picture showing Hinf I digested different MTHFR genotypes.

A large number of studies have provided a broad overview of the prevalence of the C677T polymorphism in different human population, showing that the distribution of T allele frequencies is diverse (6,16,17,22,38,48,52,53,60,61,63,66,70,71,76,78,79). These differences have been also observed between groups of different ages in the same population (36,43,72). In some populations, such

Table 1. Distribution of MTHFR genotypes and allele frequencies among Brahmin population.

Population	Genotype			Alleles		x^2 value
	CC	CT	TT	C	T	
Observed number	64	22	2	150	26	0.004
Frequency	0.73	0.25	0.023	0.852	0.148	(p=0.997)
95% CI	0.627-0.815	0.168-0.348	0.004-0.073	0.794-0.901	0.101-0.206	

Table 2. Distribution of MTHFR genotypes and allele frequencies among Rajput populations.

Population	Genotype			Alleles		x^2 value
	CC	CT	TT	C	T	
Observed number	88	25	2	201	29	0.021
Frequency	0.765	0.217	0.017	0.874	0.126	(p=0.989
95% CI	0.681-0.836	0.147-0.299	0.03-0.05	0.826-0.913	0.0877-0.174	

Table 3. Frequencies of mutant genotypes (CT and TT) and allele (T) frequencies reported in different Indian Studies.

Study	State/Region	Sample size	Frequecy of CT genotype	Frequecy of TT genotype	T allele Frequency
Mukherjee et al., 2002 [54]	Maharashtra	205	0.307	0.024	0.178
Angeline et al., 2004 [52]	Tamil Nadu	20	0.15	0.0	0.075
Radha Rama Devi et al., 2004 [59]	Andhra Pradesh	420	0.178	0.012	0.101
Kumar et al., 2005 [60]	Delhi	199	0.236	0.025	0.148
Singh et al., 2005 [61]	UP	200	0.185	0.0	0.093
Panigrahi et al., 2006[62]	Delhi	60	0.067	0.0	0.033
Rai et al., 2006 [63]	UP	165	0.236	0.012	0.13
Dalal et al., 2007[64]	UP	60	0.2	0.0	0.15
Bhat et al.,2008[67]	Kashmir	100	0.33	0.83	0.215
Kohli et al., 2008[68]	Delhi	109	0.293	0.055	0.202
Poduri et al.,2008[69]	Chandigarh	103	0.174	0.0	0.09
Saraswathy et al.,2008 [70]	Haryana/ Ahir	38	0.053	0.0	0.03
	Jat	43	0.023	0.046	0.06
Shekari et al., 2008[71]	Chandigarh	200	0.34	0.035	0.205
Ali et al., 2009[72]	UP	214	0.168	0.009	0.093
Angeline et al., 2009[58]	Tamil Nadu	100	0.2	0.0	0.1
Cyril et al., 2009[11]	Karnataka	120	0.0	0.0	0.0
Sadananda Adiga et al.,2010[75]	Karnataka	99	0.141	0.0	0.070
Harisha et al., 2010 [73]	Karnataka	102	0.137	0.009	0.078
Lakshmi et al.,2010[17]	Andhra Pradesh	280	0.175	0.0	0.087
Naushad and Radha Rama Devi,2010[74]	Andhra Pradesh	80	0.2	0.0	0.1
Deb et al., 2011 [76]	Delhi	222	0.288	0.040	0.185
Godbole et al., 2011[13]	Gujarat/ Maharashtra/ Andhra Pradesh/ Tamil Nadu	684	0.231	0.007	0.123
Rai et al., 2011[77]	UP	139	0.07	0.008	0.039

as Toscanians in Italy [1] and Mexicans, the homozygous mutated genotype (TT) has reached frequencies greater than 30%. On the other hand, in Africans the frequency of the TT genotype is very low, less than 1% (23,567). The highest T allele frequency (54%) is reported in state of Yucatan, Mexico [20]. The frequency of 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 populations of Asia. The frequency of the 677T allele in Africa is 0.06 and 0.1 in African Americans. Very scarce data from Asian countries are available like- Japan (40,46,61), Mongolia (66), Thailand (3), China (48,66,75,81), Hong Kong (66), Indonesia (63), Sri Lanka (66) and some reports from India. Frequency of mutant T allele is reported to be 0.14 in the Thais and percentages of homozygotes (TT) and heterozygotes (CT) genotypes are reported to be 1.4% and 25.6 % respectively (3), which are comparable with the result of present study. However, comparatively higher T allele frequency is reported from other Asian populations viz-Japanese (0.33) and Chinese (0.28), similar to Caucasians (40,81). Thus a variation in the C677T MTHFR genotype distribution and T allele frequency is not found only among the various ethnic groups of the world but also among the different populations of Asia (41).

Several studies were published from India regarding C677T polymorphism during past decade (2,4,5,9,11 -13

,15,19,24,31,33,35,41,42,44,45,47,49,50,54,55,64,68,69) (Table 3). Except four studies (9,50,55,64), all these twenty one studies are case-control studies. In case-controls studies, controls are selected on the basis of good health and folate and vitamin B-12 status might be higher in these controls. Other four studies (9,50,55,64) in which subjects were randomly selected from specific caste populations and represented the prevalence of true T allele frequency in different Indian population and except one study (9) low prevalence of T allele was observed. The lowest T allele frequency (0.03) was observed by Saraswathy et al (64) in Ahir caste group of Haryana and the highest frequency (0.215) was reported in Kashmiri population by Bhat et al (9]) which might be due to folate and vitamin B-12 rich non-vegetarian food habits of Kashmiri population (Table 3).

A possible explanation for low prevalence of C677T mutation in Indian population is due to genotype-specific fetal survival. It may be postulated that periconceptional supplementation of folic acid can lead to the genetic selection of T allele or permit the survival of deleterious genotype (TT). Low periconceptional folate may result in mortality of fetuses having T allele. It is well reported that homozygosity of C677T mutation is associated with a two to three fold increased risk of recurrent pregnancy loss, probably because of hyperhomocysteinemia in the the absence of folate supplementation. Radha Rama Devi et al. (50) have observed the impact of MTHFR C677T mutation on fetal viability and reported the high mortality in fetuses with T allele. Similarly several other studies conducted in different populations have also concluded that increase in mutant T allele causes decrease in fetal viability (26).

Indian population has been reported to be generally high in homocysteine, low in folate/vitamin levels, and low in T allele frequency (34,51,59). In folate/vitamins depleted populations, frequency of T allele is low because of it is under selection pressure and when present, adds to disease susceptibility. This is also a possible reason why in Europe, where these micronutrients are adequately supplemented, MTHFR C677T polymorphism occurs at a much higher frequency in general populations with much lower risk to disorders. In Indian populations, homozygous MTHFR CC677 might be physiologically protective, having a selective advantage while the T allele would put the individual at disease risk. Multiple factors could be the reason for the difference between our observations in this study and results reported by others. Those include differences in ethnicity, gene-gene interaction and gene-environmental interaction. MTHFR mutation homozygosity conferred a survival advantage in populations with adequate folic acid consumption, which may explain the observed variability in its prevalence in different populations. We suggest that there is removal of T allele (or selection of C allele) during early stages of embryonic development because of a low folate and vitamin B-12 periconceptional status in Indian pregnant women.

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