# MTHFR C677T Polymorphism among Meiteis of Manipur (India)

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**Background:** The enzyme MTHFR catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the reaction converting homocysteine to methionine. Mutation at MTHFR gene (C677T) has been implicated in the pathogenesis of common complex diseases such as thrombosis, hypertension, stroke, myocardial infarction, and recurrent pregnancy loss across world populations.

**Objective:** We wanted to explore C677T mutation among Meiteis of Manipur to generate baseline data and to gain information that could be used in disease prevention programs.

**Methods:** A total of 1142 (625 males and 517 females) unrelated individuals aged 35 to 75 years were involved in the study. 1098 samples could be genotyped for MTHFR C677T polymorphism.

**Results:** MTHFR C677T was found to be polymorphic in the Meitei population studied. Around 30% of individuals are carrying the mutant allele either in heterozygous or homozygous condition with T allele frequency of .16.

**Conclusion:** Among study participants, those with T allele frequency of .16 may be predisposed to complex diseases, if their active lifestyles are shifted to sedentary lifestyles. Relatively lower frequency of T allele among individuals of younger age (though not significant) is indicative of selective disadvantage of this allele in the recent years. (*Ethn Dis*. 2013;23[3]:379–381)

**Key Words:** Methylenetetrahydrofolate Reductase, Meiteis, Polymorphism

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## Introduction

The enzyme MTHFR (methylenetetrahydrofolate reductase) catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the reaction that converts homocysteine to methionine. The gene encoding MTHFR is located at 1p36.3.[1] Several polymorphisms in the MTHFR gene have been reported to be associated with various pathological conditions. Frosst et al described a C to T substitution at nucleotide 677 that converts an alanine to a valine residue, which is responsible for the synthesis of thermolabile form of MTHFR.[2] This polymorphism, which decreases the activity of the enzyme and leads to hyperhomocysteinemia and possible increased risk of coronary artery disease,[3] has been reported to be common in many different populations.

In addition to the genetic factors, various environmental factors, including diet, are known to influence the levels of homocysteine.[4] Folate and vitamin B12 are the critical nutritional factors, a deficiency of which results in hyperhomocysteinemia.

The 677T mutation is related to various disorders, such as thrombosis, hypertension, stroke and coronary heart disease[5–8]; yet the T allele frequency differs greatly among ethnic groups of the world. The frequency of the T allele is found to be highest among European populations (64.3%)[9] and lowest among African populations.[10]

Our study sought to screen the Meitei population of Manipur to gain baseline data on the frequency distribution of the 677T allele for such clinically important gene polymorphism, which is needed for proper counseling strategies.

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## Materials and Methods

A total of 1142 random blood samples were collected from 625 males and 517 females, aged 35–75 years and who were unrelated up to first cousin. The participants belonged to the Meitei community living in four valley districts of Manipur, India; Imphal East, Imphal West, Thoubal and Bishnupur districts. Informed written consent was obtained from all participants before collecting the samples. Ethical clearance was obtained from the Departmental Ethical Committee, Department of Anthropology University of Delhi. Within Manipur, Meiteis comprise 60% of the total population. They are mostly a non-vegetarian, Tibeto-Burman language speaking Mongoloid population who practice clan exogamy and are physically well built. They are primarily agriculturalists, although they have been shifted to other occupations like business in recent times.

Genomic DNA was isolated using the salting out method.[11] MTHFR C677T polymorphism was analyzed using the standard protocol.[12] A total of 1098 DNA samples could be genotyped. Allele frequencies were calculated by gene counting method using POPGENE software. A $\chi^2$ test was performed

**Table 1. Distribution of MTHFR C677T genotype and allele frequencies among Meiteis of Manipur**

| Single Nucleotide Polymorphism | Total Sample Size | Observed Genotypic Count | | Allele Frequency C | Allele Frequency T | $P^a$ |
|---|---|---|---|---|---|---|
| MTHFR C677T | 1098 | CC | 776 | .84 | .16 | .98 |
| | | CT | 294 | | | |
| | | TT | 28 | | | |

[a] Significance level at $P<.05$.

to test Hardy-Weinberg equilibrium and also for age-wise comparison of genotypic frequencies of MTHFR C677T polymorphism. Statistical significance was set 5%.

## RESULTS

The results of the analyses can be found in Tables 1 and 2. The MTHFR gene, with respect to C677T polymorphism, was found to be polymorphic in the study population with the frequency of T allele being .16; also the population is in Hardy-Weinberg equilibrium (Table 1).

The frequency of CT heterozygotes and mutant TT homozygotes were found to be higher among the older (≥50 years) vs younger (<50 years) participants, (CT: 28.09% vs 25.25%, TT: 3.21% vs 1.77%, respectively) and the frequency of T allele was 17% among those ≥50 years vs 14% among younger participants. However, the difference between the two age groups with respect to MTHFR gene polymorphism was not found to be statistically significant ($P=.15$) (Table 2). A lower frequency of T allele among the younger group is indicative of a selective disadvantage of the allele in the population in recent years.

## DISCUSSION

The T allele frequency of MTHFR C677T is found to vary across world populations. The frequency of the T allele of the MTHFR C677T polymorphism reaches frequencies as high as 64.3% among European populations[9] while registering low frequencies in African populations.[10] Among Indian populations, Caucasian populations of North India (3% among Ahirs to 23.75% among Sindhi)[13,14] and Mongoloid populations of North East India (0% among Koms to 23.1% among Lothas)[14,15] including our study population (16%) are found to have relatively higher frequency of T allele as compared to Central (0% among Munda, 2.22% among Oraon)[14] and South Indian (4.29% among Nayakpod to 11.7% among Thoti)[14] Dravidian populations. In comparison with the Mongoloid populations of Southeast Asia (16.7% among Japanese to as high as 55.2% among Chinese),[16,17] the T allele frequency of our study population was found to be low. In general, Mongoloid populations of India show very high frequencies of the T allele as compared to other non-Mongoloid populations of India.[14,15] However, they show a relatively lower frequency of T allele when compared with the Mongoloid population of Southeast Asia.[16,17] Furthermore, T allele frequency among these Mongoloid populations was found lower than that of European population.[9] Considering the higher frequencies of T allele observed in European populations, people have hypothesized that the T allele originated in Europe in the late stage of human evolution and later spread to various parts of the world.[14] T allele is found to be implicated in various complex disorders as MTHFR is involved in a metabolic pathway, that, if blocked, results in hyperhomocysteinemia, which could lead to many pathological conditions. However, some studies report a positive association of MTHFR T allele with various diseases[18] while some report no association.[19] Undoubtedly, the T allele is increasing in populations, which goes against selective disadvantage that it should have, when associated with various diseases. Moreover, these complex diseases often set in later life (ie, after reproductive age) and, by then, the allele is already passed on to the next generation, which could be partly responsible for the increased

**Table 2. Age-wise distribution of MTHFR C677T genotype and allele frequencies among Meiteis of Manipur**

| Single Nucleotide Polymorphism | Genotypes | <50 years *n* | <50 years % | ≥50 years *n* | ≥50 years % | Allele Frequency <50 years C | Allele Frequency <50 years T | Allele Frequency ≥50 years C | Allele Frequency ≥50 years T | $P^a$ |
|---|---|---|---|---|---|---|---|---|---|---|
| MTHFR C677T | CC | 370 | 72.98 | 406 | 68.7 | .86 | .14 | .83 | .17 | .15 |
| | CT | 128 | 25.25 | 166 | 28.09 | | | | | |
| | TT | 9 | 1.77 | 19 | 3.21 | | | | | |
| | Total | 507 | 100 | 591 | 100 | | | | | |

[a] Significance level at $P<.05$.

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frequency of the T allele. Further, recent studies have also reported a heterozygous advantage for this allele, which would be another reason for the increase of this allele in various populations.

In addition to the relatively high mutant allele frequency in our study population, it is likely that the more sedentary lifestyles they are beginning to adopt will further put them at risk of complex diseases as proposed by Murry et al.[15] Mayor-Olea et al[20] reviewed reported data on individuals born in the southern Spain in four groups according to birth date (1900 to 1925; 1926 to 1950; 1951 to 1975; 1976 to 2000); they found an increase in the frequency of T allele and TT genotype in those born in the last quarter of the century. In our study population, the T allele frequency was found to be slightly lower in the younger age group (<50 years) than in the older age group (≥50 years); however, the difference was not statistically significant. This is suggestive of the selective disadvantage of this allele in recent years. Thus, data generated in our study offers more information on possible causes of complex diseases among the Meiteis. Health planners can use this information to develop health programming to encourage physical activity and a diet richer in foods with vitamin B12 and folic acid for those at-risk.

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## AUTHOR CONTRIBUTIONS

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