

## Original Article

# Genotype and Allele Frequency of Methylenetetrahydrofolate Reductase 677CT mutation in Female Arabs residing in the United Arab Emirates

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### Abstract

The MTHFR gene (Methylenetetrahydrofolate reductase), responsible for encoding the MTHFR enzyme, is vital for the body's methylation processes, which are crucial for DNA synthesis, repair, and overall metabolic functions. Previous studies have shown that mutations in the MTHFR gene are associated with various diseases, including neural tube defects, male infertility, type II diabetes mellitus, cardiovascular diseases, and certain cancers. Additionally, abnormal methylation of the MTHFR gene can suppress other important genes such as methionine synthase, thymidylate synthase, choline kinase, and folate receptor genes. This suppression can result in a deficiency of the MTHFR enzyme, essential for proper cellular function, especially when a 677CT (C to T) transition occurs, leading to reduced enzyme activity. Despite the known implications of MTHFR gene mutations, no studies have probed these mutations in the female Arab population. To fill this gap, a pilot study was conducted to determine the prevalence of the MTHFR C677T gene mutation among 45 healthy Arab individuals in the United Arab Emirates. The study used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method to detect the mutation. The study found that the mutated T allele had a 6% occurrence rate in the female Arab population. The genotype frequencies were 86.6% for the CC genotype, 13.3% for the CT genotype, and 0% for the TT genotype, indicating a relatively low prevalence of the MTHFR C677T polymorphism in Arab women. These findings provide preliminary data that can form the basis for further research on MTHFR gene mutations in this population, potentially enhancing the understanding and management of related health conditions.

**Keywords:** Methylenetetrahydrofolate reductase (MTHFR), genotype frequency, allele frequency, hyperhomocysteinemia, mutations

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### Introduction:

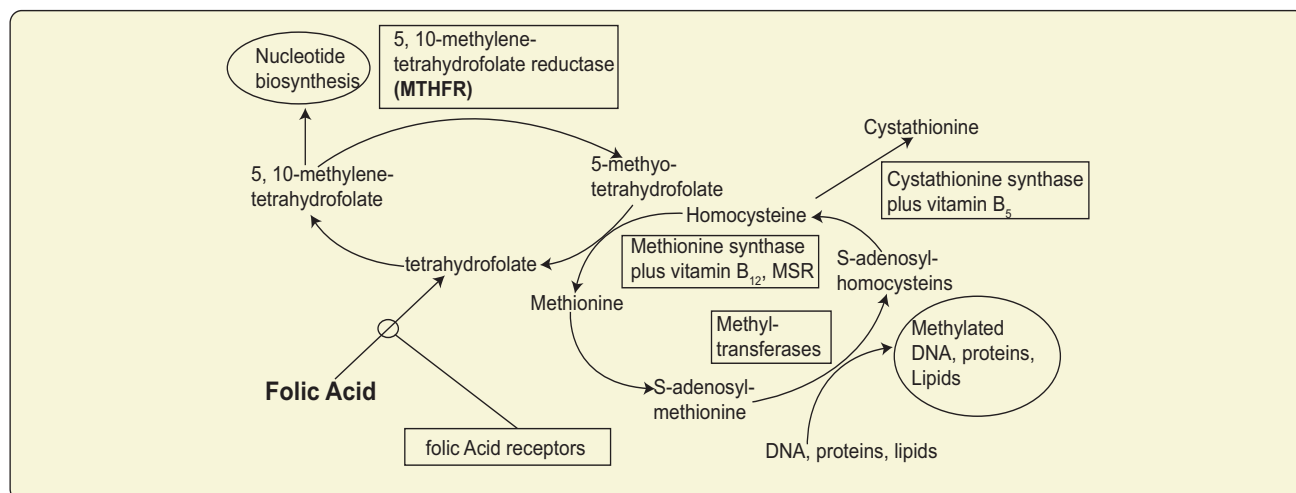
In humans, the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHFR) to 5-methyltetrahydrofolate (5-MTHFR) is facilitated by the vital enzyme called methylenetetrahydrofolate reductase<sup>2,9</sup> (MTHFR) (Figure 1). 5-MTHFR, a co-substrate, is a form of folate necessary for methionine production as it converts homocysteine to methionine by

acting as a methyl donor.<sup>1</sup> There is subsequent conversion of methionine to a critical methyl donor, known as S-adenosylmethionine, which has important roles in proteins, nucleic acids, & other biological compounds-involved reactions. As a result, methionine plays an important role in vital bodily processes such as gene expression regulation and neurotransmitter production. Additionally, the MTHFR enzyme plays an important role in folate metabolism, a process important for RNA, DNA, and protein production.<sup>9</sup> The differences found in the DNA sequence of the MTHFR gene, encoding the methylenetetrahydrofolate reductase enzyme, are referred to as the MTHFR gene variation. The long 2.2 kb MTHFR gene, has 11 exons and is found on chromosome number 1 at position p36.3.<sup>1,14</sup>

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**Figure 1:** MTHFR-involved metabolic pathways (MSR: Methionine synthase reductase) (Watkins D and Rosenblatt DS, J Inherit Metab Dis. 2012).

The MTHFR gene contains two prevalent polymorphic variants that encode for the MTHFR enzyme. These commonly known variants are the 665C→T (p. Ala222Val) and c.1286A→C (p. Glu429Ala) variations, known as C667T and A1298C respectively. These variants are missense changes that are associated with decreased enzyme activity. Out of these variants, the MTHFR C677T mutation stands out as the most frequently detected mutation in the MTHFR gene.<sup>1,9</sup>

Since it has come to be known that the 677C→T (A222V) variant is the most prevalent genetic cause of hyperhomocysteinemia, it has gained particular attention.<sup>3</sup> The C677T point mutation, located at position 677 nucleotide of exon 4, causes cytosine nucleotide to convert to thymine nucleotide. The consequence of this mutation is the replacement of the amino acid alanine with valine, leading to a substantial decrease of around 70% in the activity of the MTHFR enzyme. This variant has garnered significant focus due to its prevalence in causing hyperhomocysteinemia, and its potential involvement in various complex diseases associated with abnormalities in the metabolism of homocysteine.<sup>3</sup> A thermolabile enzyme is encoded by the T variant which leads to about 30% of activity in the homozygous (TT) state and 65% in the heterozygous (CT) state.<sup>3,9</sup>

The variant is prevalent in the general population among Caucasian populations, with homozygosity (TT) rates ranging from 6% to 14%. However, Africans and individuals outside of Africa (such as in Brazil and the United States) have lower homozygosity frequencies for the 677T allele, typically below 2%. Conversely, the 677T allele is notably widespread among individuals of Hispanic descent. Research

conducted on Hispanic origin in Colombia and California, who have a strong southern Mediterranean ancestry, have reported homozygosity rates (%TT) of 21% and 18% respectively, indicating a significant presence of the variant.<sup>1,16</sup>

Ethnic and geographic variation can be responsible for the difference in C677T allele frequency; in California, Hispanics are reported as having high allele frequency and Afro-Americans are associated with low frequency. Additionally, there's marked variation in C677T homozygote frequency between different populations. Hispanics, Colombians, and American Indians in Brazil have the highest frequency of more than 20% as opposed to less than 2% of the variant genotype found in the African population. The frequency can range from 8-20% among the white populations in Australia, Europe, and North America. Interestingly, drift can be noticed in the homozygote variant occurrence from Northern to Southern Europe<sup>8</sup>.

The MTHFR A1298C mutation is present in approximately 7-12% of the population in North America, Europe, and Australia.<sup>3</sup> However, this mutation is less prevalent among Hispanics, Chinese, and Asians.<sup>5,7</sup> Individuals who are homozygous for MTHFR A1298C, are predicted that their enzyme function is reduced to about 60% of the native form. The term “double heterozygous” is employed to refer to individuals who possess an abnormal MTHFR C677T gene along with an abnormal MTHFR A1298C gene. This combination can also result in a reduction of enzyme function.<sup>2</sup>

Individuals who carry a genetic variation in the MTHFR gene show decreased levels of 5-MTHFR, leading to the increased amount of homocysteine. The elevation of this

amino acid has been associated with an elevated risk of cardiovascular and neurological disorders such as dementia and Alzheimer's disease.<sup>2</sup> However, this effect can be compensated by supplementing with folate. Folate serves as an essential co-factor needed in converting homocysteine into methionine, thereby compensating for the reduced enzyme function caused by the genetic variation.

A range of complex diseases might occur due to the lower enzyme activity occurring in individuals with MTHFR gene variation including coronary artery disease, recurrent miscarriages, & neural tube defects due to the mentioned hyperhomocysteinemia.<sup>2,4,15</sup> A few studies have shown that families with neural tube defects had higher plasma total homocysteine levels. Following its initial discovery, the MTHFR variant was swiftly identified as the primary genetic risk factor for defects in the neural tube.<sup>3</sup> Consequently, women who carry MTHFR gene variations may face an increased likelihood of having a child with a neural tube defect, especially when combined with low levels of folate.

Mutation in the MTHFR gene has also been associated with conditions such as Down's syndrome, bladder cancer, breast cancer, colon cancer, depression, leukemia, glaucoma, schizophrenia, thrombosis, vascular disease, and migraine.<sup>4,8,18</sup>

In the Turkish population, Yigit et al. conducted a study and found a notable correlation between the C677T mutation in the MTHFR gene and the incidence of diabetic neuropathy. Furthermore, their research also revealed a link between the MTHFR C677T mutation and the presence of retinopathy in individuals with diabetic neuropathy.<sup>17</sup>

In a recent study by Tongboonchoo et al., the association between the MTHFR C677T polymorphism and osteoporosis in postmenopausal women was seen.<sup>19</sup> The incidence of osteoporosis and osteopenia increases in postmenopausal indicating the association between MTHFR gene and bone mineral density regulation. Moreover, Zidan et al. focused on the link between congenital heart diseases (CHD) and polymorphisms in MTHFR A1298C mutation in children of Egyptian descent and their mothers.<sup>20</sup>

Additionally, the TT genotype of the MTHFR-C677T polymorphic gene significantly influenced homocysteine levels in Malaysian patients with ischemic stroke.<sup>21</sup>

Subsequent investigations have been done to examine the MTHFR C677T mutation occurrence in females experiencing infertility as well as those who have experienced failed implantation. However, the results of these studies have yielded controversial findings. According to studies conducted, 49.2% of the fertile women and 58.5% of the

infertile women had the MTHFR C677T mutation, a difference that was not statistically significant.<sup>24</sup>

Another study reported that women carrying the 677T variant had a slightly lower number of oocytes, although the observation was statistically insignificant since a limited number of cycles were examined. The results also indicated that a female's folate metabolism influences the synthesis of steroid hormones in her granulosa cells (GCs). The data obtained supports the hypothesis that mutations in the MTHFR C677T impact oestradiol production within individual GCs and follicles, affecting both the ability of tertiary follicles to generate oestradiol and the number of follicles present.<sup>22,23,25</sup> Research suggests that women with MTHFR C677T have ovaries that produce less estrogen and are less responsive to the follicle-stimulating hormone (FSH) during ovulation.<sup>25,26,27,29</sup> Women who have C677T or the combination of C677T and A1298C are more likely to have recurrent implantation failure during IVF procedures<sup>28</sup>.

As mentioned previously, consuming adequate amount of folate in the diet can help individuals with MTHFR gene variations to manage their health risks. Leafy greens, citrus fruits, and beans can be a source of good amount of folate. As mentioned previously, folic acid supplements can be beneficial as well.<sup>10,11,12,13</sup> Appropriate dietary and lifestyle changes can be made if genetic testing is done to identify an individual's specific MTHFR gene variant. This allows the risk management to be tailored accordingly. Routine clinical practice does not warrant the use of MTHFR mutation testing for any specific patient group. The ACMG (American College of Medical Genetics) advises against ordering this genetic test as part of clinical evaluations aimed at assessing the risk of blood clots or recurrent abortions (pregnancy loss).<sup>6,13</sup>

Dietary habits and lifestyle changes in the United Arab Emirates (UAE) have led to a rise in patients with complex diseases. Given the role of the MTHFR gene in several complex conditions, such as female infertility, and its significant variation across different ethnic and regional populations worldwide, this study aimed to investigate the heterogeneity of the MTHFR gene among the female Arab population living in the United Arab Emirates.

### Objective

- To identify the pattern of genetic variations in the MTHFR gene among healthy female individuals of Arab descent living in the United Arab Emirates.
- To determine the specific types of MTHFR C677T single nucleotide polymorphisms present within the Arab population residing in the United Arab Emirates.

### Significance of the Study

The MTHFR gene provides the instructions for the body to produce the MTHFR protein, which is essential for the absorption of folate. Folate is necessary for the body to create DNA and modify proteins. A deficiency in MTHFR leads to the accumulation of folic acid, as it cannot be converted to methyl folate due to the enzyme deficiency. This results in severe homocystinuria, with only 20% of the enzyme being active, which clinically manifests as severe complex diseases and infertility.

Determining whether a typical MTHFR gene mutation is causing elevated homocysteine levels in the blood may help reduce the risk of developing infertility and other complex diseases later in life. Understanding this gene mutation's impact is crucial for early intervention and prevention strategies.

### Materials and Methods

Blood samples from female Arab volunteers residing in the United Arab Emirates (UAE) were collected for the pilot study. Ethical approval for the study was obtained from the Ethical Committee of Dubai Pharmacy College for Girls (REC/UG/2022/05). Participants gave written consent and were informed of their right to withdraw from the study at any stage. Volunteers did not receive any compensation for their participation, and for student volunteers, it didn't impact their academic grades. This cross-sectional pilot study was conducted from October 2022 to May 2023.

### Sampling Method

Subjects for this preliminary study were selected using a convenience sampling method, comprising 45 healthy women from the general Arabian Gulf population residing in the United Arab Emirates. The sample size was determined based on guidelines from prior literature on sample size and statistical power calculation in genetic association studies to ensure sufficient number for statistical analysis.<sup>20</sup>

- Individuals above the age of 18 years.
- Volunteers who expressed their willingness to participate in the study.
- Individuals available during the specified timeframe.

### Exclusion Criteria

- Individuals below the age of 18 years were excluded from the study sample.

### Genomic DNA Extraction

Blood samples were collected in sterile vacutainers containing EDTA and stored at 4°C until DNA extraction. Genomic DNA purification was carried out using the Wizard

Genomic DNA Purification Kit (Promega, Madison, USA). The process involved isolating genomic DNA from 1 mL of whole blood.

### DNA Purity - Spectrophotometric Method

A spectrophotometer was used to assess the quality of the extracted DNA. Initially, 50 µL of TE buffer was introduced into a quartz cuvette for auto-zero correction. To this, 48 µL of TE buffer and 2 µL of the DNA sample were added to the cuvette. Absorbance measurements were taken at wavelengths of 260 nm and 280 nm. The absorbance at 260 nm indicated the DNA concentration, while the 260/280 ratio indicated DNA purity. DNA samples were deemed suitable for PCR analysis if the absorbance ratio was between 1.7 and 1.9. Samples with ratios below 1.7 underwent additional precipitation to achieve the desired absorbance.

### PCR-RFLP Analysis for MTHFR C677T Genotyping

PCR-RFLP analysis, involving *HinfI* digestion, was performed to genotype the MTHFR C677T mutation. The primer sequences used were:

- Forward: 5'-TGA AGG AGA AGG TGT CTG GGG GA-3'
- Reverse: 5'-AGG ACG GTG CGG TGA GAG TG-3'

The 198 bp amplicon is digested into 175 and 23 bp fragments due to the introduction of a new *HinfI* restriction site by the C677T mutation.

In a final volume of 50 µL, the PCR mixture included:

- 1 µmol/L of each primer
- 2 units of Taq polymerase
- 25 mmol/L of MgCl<sub>2</sub>
- 0.2 mmol/L of each dNTP
- 1 µg of DNA template

The amplification was carried out using a PCR thermal cycler with the following cycling parameters:

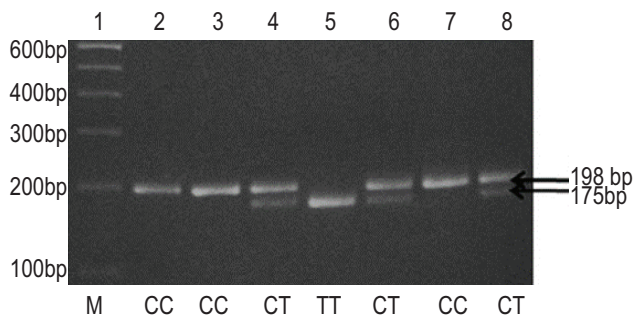
- Initial denaturation at 95°C for 5 minutes
- 35 cycles of 95°C for 45 seconds, 55°C for 1 minute, and 72°C for 45 seconds
- Final extension at 72°C for 10 minutes

The 198 bp PCR product (10 µL) was digested with the restriction enzyme *HinfI* at 37°C for 3 to 4 hours in the manufacturer's recommended buffer. The C to T substitution, detectable by *HinfI*, results in an Ala-to-Val residue conversion in the MTHFR coding region.<sup>22</sup>

### Gene Analysis by Gel Electrophoresis

On a 2% agarose gel, 20 µL of each reaction mixture was separated, stained with ethidium bromide, and visualized

under UV light. The alleles were designated as T (Val) and C (Ala). HinfI does not digest the 198 bp fragment from the C allele, while it digests the fragment from the T allele into 175 bp and 23 bp fragments. Subjects homozygous for the mutation had two DNA fragments of 175 bp and 23 bp. Non-homozygous subjects had a single 198 bp fragment whereas heterozygous subjects displayed three DNA fragments of 198 bp, 175 bps, and 23 bp (Figure 2).



**Figure 2:** Different MTHFR Genetic variants identified in samples.

**Statistical Analysis**

Genotype and allele frequencies were calculated using the gene counting method. The Chi-square test was employed to assess Hardy-Weinberg equilibrium. Statistical significance was confirmed at the 5% probability level.

**Results & Discussion**

**Table 1.** Genotype frequency of MTHFR C677T mutation in healthy female Arabs.

MTHFR C677T genotype	Healthy Females n=45	Genotype Frequency
CC	39 (86.6 %)	0.866*
CT	6 (13.3 %)	0.133
TT	0 (0 %)	0.0

p<0.05

Results for MTHFR Gene Polymorphism (Table 1).

In the Arab population, the homozygous C genotype was found in 86.6% of the individuals.

The heterozygous CT genotype was observed at a frequency of 13.3%.

Among the 45 healthy female individuals, the homozygous T genotype was absent, indicating a 0% occurrence of the TT homozygous mutation. This suggests the need for conducting the study with a larger sample size. A similar result was reported in a previous study conducted by Atadzhanov M et al (2014)<sup>23</sup> where the TT genotype was absent in the Zambian population.

Other studies reported contradictory findings, with Europe reporting the highest frequency of the 677T allele in the world, ranging from 24.1 to 64.3%. North America came in second (6-64.3%), followed by East Asia (2-55%), South America (2-48.7%), Asia (2.5-45%), Africa (0-35.5%), Siberia (8-31.5%), and Oceania (2.9-28.6%) (Saraswathy KN, 2012)<sup>24</sup>. It can be assumed that the allele emerged in Europe during the late stage of human evolution given the greater frequencies of 677T seen in Europe and North America. The transmission of this gene to additional regions may have been aided by migration, settlement, and colonization. In comparison to South America, which is primarily populated by indigenous people, North America, which has more European immigrants, had a higher frequency of the 677T gene. The above argument is also supported by the fact that this allele is more common in Caucasian groups than in Mongolian and African populations.

That the population is not in Hardy-Weinberg Equilibrium regarding MTHFR polymorphism, accordingly the Hardy-Weinberg, chi-square value obtained for Arabs living in the UAE was 0.2296 (Table 2). Since the ratio between observed genotype frequency and expected genotype frequency is not equal to 1, thus variations between the expected and observed values are not statistically significant therefore the population does not follow Hardy Weinberg equilibrium.

**Table 2.** MTHFR genotypes, Hardy-Weinberg expectations, and  $\chi^2$  value

Genotype			Observed frequency			Expected frequency			Observed/ Expected ratio			$\chi^2$
CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT	
39	6	0	39	6	0	39.2	5.6	0.2	0.9	1.1	0	0.2296

Our results correlated with the research done by Banerjee B & Saraswath KN (2016)<sup>8</sup> where the study aimed to identify MTHFR C677T SNP between the Bhils tribe of Udaipur, Rajasthan, and subsequently compare the results with different populations of the world. They found that their population did not follow Hardy-Weinberg Equilibrium for the MTHFR polymorphism ( $P < 0.05$ ).

**Table 3.** Allele frequencies of MTHFR C677T polymorphism in Healthy Female Arabs.

Allele/ Locus	Frequency
Allele C	0.93
Allele T	0.06

The “C” allele has an allele frequency of 0.93, while the “T” allele has an allele frequency of 0.06 (Table 3).

Our findings align with those of Banerjee B and Saraswath KN (2016)<sup>8</sup>, who investigated the MTHFR C677T SNP in the Bhils tribe of Udaipur, Rajasthan, and compared their results with those of Indian and global populations. They found that the Bhils had an allele frequency of 0.96 for the “C” allele and 0.05 for the “T” allele.

The MTHFR gene polymorphism at the C677T point mutation was identified in the healthy female Arab population. Due to the clinical significance of this gene, numerous recent studies have focused on the MTHFR gene, particularly the C677T SNP. The 677T mutation causes hyperhomocysteinemia by reducing the gene’s enzymatic activity by up to 70%. However, the administration of methyl folate can mitigate the negative effects of this mutation, along with factors such as mating habits, migration histories, and ethnic origins. Despite the known association between hyperhomocysteinemia and various complex disorders, the specific role of MTHFR in the pathophysiology of these disorders remains unclear.<sup>4</sup>

### Recommendations

To address elevated homocysteine levels, it is recommended to test the MTHFR and CBS genes. The normal plasma homocysteine level ranges from 5-12  $\mu\text{mol/L}$ . If high homocysteine levels are detected, a basic pro-methylation protocol should be implemented. This includes regular intake of TMG (betaine) 250 mg, 400 mcg folate (5-MTHF), 5 mcg Vitamin B12, 3 mg Vitamin B6, 2.4 mg Vitamin B2, and 12.5 mg Zinc. Increasing dietary folate helps produce the active form of 5-MTHF. Therefore, the diet should include folate-rich vegetables such as asparagus and broccoli, as well as brightly colored fruits like mangoes.

### Conclusion

This study investigates the MTHFR C677T polymorphism in healthy female Arabs in the UAE, finding a high frequency of the homozygous C genotype (86.6%) and a significant presence of the heterozygous CT genotype (13.3%), with no instances of the homozygous T genotype. These results indicate a low occurrence of the T allele in this population.

The clinical significance of the MTHFR C677T SNP is linked to hyperhomocysteinemia, associated with cardiovascular diseases, neural tube defects, and infertility. The 677T mutation can reduce MTHFR enzyme activity by up to 70%, increasing homocysteine levels, highlighting the need for genetic screening for early intervention.

The study suggests dietary and supplemental interventions, such as TMG, folate, Vitamin B12, B6, B2, and Zinc, to manage elevated homocysteine levels. Further research with larger samples is needed to understand the full impact of the MTHFR C677T polymorphism in different populations.

### Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

### Author Contributions

All authors have contributed significantly to the research and writing of the paper in addition to formatting the main manuscript. This fulfills the current JOM criteria for Authorship.

### Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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