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# The Frequency of C677T Methylenetetrahydrofolate Reductase (*MTHFR*) Polymorphism in Southern East Bosnian Population

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## Abstract

The C677T *MTHFR* polymorphism is distributed widely among ethnics populations and showing a high heterogeneity. The fact that there were no published data on the prevalence of C677T *MTHFR* polymorphism in Bosnia and Herzegovina prompted us to determine its prevalence in sample of Bosnian population.

Two hundred and seven unrelated, apparently healthy subjects from Southern east Bosnia were included in study. Genotyping of C677T *MTHFR* polymorphism was done using polymerase chain reaction (PCR) followed by restriction digestion (RFLP) with HinfI enzyme.

Out of 207 healthy subjects, 44.44% were heterozygous and 11.11% were homozygous for C677T *MTHFR* polymorphism. Allele and genotype frequency of *MTHFR* C677T did not differ between males and females carriers ($X^2$=0.87; df=1; P=0.350).This study is the first to report frequency of C677T polymorphism in healthy Bosnian population. Frequency of the T allele and C677T *MTHFR* genotype observed in this study is consistent with the data from literature.

**Keywords:** C677T Methylenetetrahydrofolate Reductase (*MTHFR*) polymorphism; PCR; RFLP

## Introduction

Methylenetetrahydrofolate Reductase (*MTHFR*) enzyme catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, and the methyl donor for the conversion of homocysteine to methionine [1].

The 5, 10-methylenetetrahydrofolate reductase (*MTHFR*) gene is located on chromosome 1 at 1p36.3. The complementary DNA sequence is 2.2 kilo bases long and consists of 11 exons [2]. Genetics polymorphism in the *MTHFR* gene are well established, the most extensively studied of which is C677T single-nucleotide polymorphism (SNP) [3]. The C677T SNP results in a missense mutation that converts a cytosine (C) into thymine (T) which is leading to substitution of valine for alanine at position 222 of the *MTHFR* enzyme, causing the synthesis of a thermo labile enzyme with a 50% reduction activity [4-6].

Reduced *MTHFR* enzyme activity is subsequently followed by increases in circulating homocysteine levels hyperhomocysteinemia) [7]. Hyperhomocysteinemia now is recognized as an independent risk factor for vascular diseases [8] and defects of the neural tube [9].

The T677 allele is distributed widely among populations showing a high heterogeneity [10]. Its frequency varies in different geographical regions and ethical groups. A number of studies have reported the frequencies of C677T in European and American Caucasian populations.

The fact that there were no published data on the prevalence of C677T *MTHFR* polymorphism in Bosnia and Herzegovina prompted us to determine its prevalence in healthy subjects in Sotherneast Bosnian population.

## Materials and Methods

### Subjects

We studied a total of 207 (102 man and 105 women) unrelated, apparently healthy subjects from Southern east Bosnia. Their mean age was 45.62 years (range 18-84) at time of blood sampling.

All subjects were fully informed about study protocol and have consented to participate in the study by signing the written consent. The study was approved by the Ethics Committee on Human Research of University Clinical Center Tuzla.

### Methods

The DNA was isolated from EDTA anticoagulated whole blood (Vacuatainer Becton Dickinson, Meylan Cedex, France) using the commercial Flexi Gene (250) Isolation Kit (QIAGEN, GmbH, Hilden, Germany). The C677T *MTHFR* polymorphism was genotyped by polymerase chain reaction followed by restriction digestion (PCR-RFLP). A 25 µL PCR reaction was performed by using 1µL genomic DNA, 10×PCR Buffer, dNTP (2,5mM), $MgCl_2$ (1, 5 mM), 5'-TGA AGG AGA ACG TGT CTG CGG GA-3' forward primer and 5'-AGG ACG GTG CGG TGA GAG TG-3' reverse primer (Eurofins MWG Operon, Ebensburg, Germany) and Taq DNA polymerase (5U/µL) (Applied Biosystems by Roche Molecular systems Inc, New Jersey, The USA).

The PCR amplification cycles were modified as follows: 5 min initial at 95°C, followed by 30 cycles of 30 sec of denaturation at 95°C, 30 sec of annealing at 60°C and 60 sec of extension at 72°C in an Applied Biosistems Thermal Cycler (Applied Biosystems by Roche Molecular systems Inc, New Jersey, The USA) apparatus.

The Ala223val substitution, due to the C677T transition in the

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*MTHFR* gene, creates an additional HinfI restriction site in the PCR amplified fragment as described Frost et al. [6] which is detected by digestion of the 198 bp PCR product, generating 23 bp and 175 bp fragments in homozygous (genotype TT). Genotype CC is characterized by the presence of a 198 bp fragment, and genotype CT characterized by the presence of two fragments, on with 198 bp and other with 175 bp. Restriction fragments were subjected to 4% agarose gel electrophoresis (Figure 1).

### Statistical analysis

Statistical analyses was accomplished by using MedCalc 9.6.2.0 statistical software package The frequencies of T and C alleles and genotypes among gender in Southern east Bosnian population were compared by a chi-squared test. The difference in the prevalence variant C677T mutation is statistically significant if P<0.05.

## Results

Two hundred and seven healthy blood donors (102 males and 105 females) representing the Southern east Bosnian population were tested for C677T *MTHFR* polymorphism. The frequencies of genotypes distribution in study group are presented in Table 1. Of the 207 subjects 41.15% (males) and 46.66% (females) had wild type genotype. Among carriers of C677T *MTHFR* polymorphism 48.03% (males) and 40.95% (females) were found to be heterozygous. Of 102 male subjects 10.78% were homozygous for C677T *MTHFR* polymorphism. Similar frequencies for homozygous genotype of C677T *MTHFR* polymorphism were observed in female's subjects (11.42%). We did not find statistically significant differences in the percentage distributions of C677T *MTHFR* polymorphism between males and females carriers in Southern east Bosnian population ($X^2$=0.87; df=1; P=0.350). The distribution of C and T alleles among males and females carriers of C677T *MTHFR* polymorphism are shown in Table 2. The frequency of normal C allele was 0.68% in male's subjects and 0.71% in female's subjects. The frequency of T allele in both subjects was similar (0.32% males and 0.29% females). The differences in distribution of C and T alleles among males and females in study group was not observed ($X^2$=0.428; df=1; P=0.512).

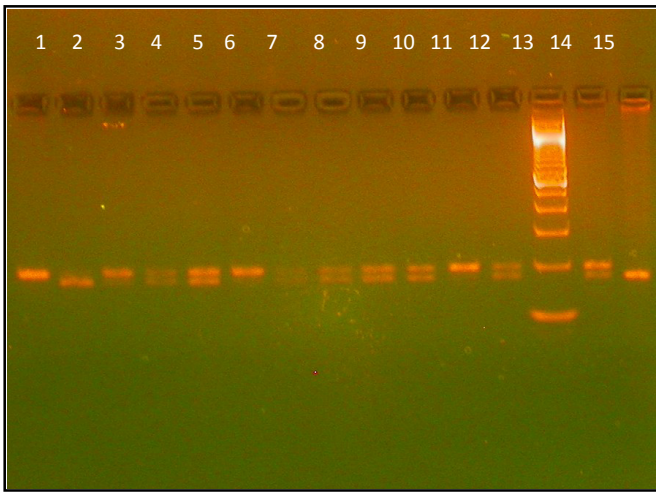


**Figure 1:** Detection of genotypes of the C677T MTHFR polymorphism by HinfI restriction analysis.
Lines 1, 6 and 11 represents three subjects with normal genotype (198 bp fragment only), whereas lines 3, 4, 5, 7, 8, 9, 20, 12 and 13 show digested fragments (198 and 175 bp) from nine subjects. Lines 2 and 15 represents two homozygous carriers. The molecular weight marker used is 100 bp ladder (Newe England Biolab, The UK) in line 13.
On the figure 1 are presented genotype for C677T MTHFR polymorphism after subjecting to 4% agarose gel electrophoresis.

| | Wild genotype CC | Heterozygous CT | Homozygous TT |
|---|---|---|---|
| Males | 42 (41.17) | 49 (48.03) | 11 (10.78) |
| Females | 49 (46.66) | 43 (40.95) | 12 (11.42) |
| **Total** | **91 (43.96)** | **92 (44.44)** | **23 (11.11)** |

**Table 1:** The frequencies of genotypes of C677T *MTHFR* polymorphism in study group.

| | Males | | Females | |
|---|---|---|---|---|
| | N | % | N | % |
| Allele C | 133 | 0.68 | 141 | 0.71 |
| Allele T | 60 | 0.32 | 55 | 0.29 |

**Table 2:** The frequencies of C and T alleles among males and females carriers of C677T *MTHFR* polymorphism.

## Discussion

Although over the last few years C677T *MTHFR* polymorphism has been widely investigated, the results of this study are the first data on the frequency of C677T polymorphism in healthy representing Southern east Bosnian population. Population genetics studies are one way to highlight geographical and ethnic differences that suggest evolutionary pressures generated by environmental factors. Such possible pressures on the nutritional environment remain speculative in the case of *MTHFR* polymorphisms [11]. The frequency of T allele in different populations ranges from 0.06 to 0.59 and the frequency if the TT genotype ranges from 0.00 to 35%.

According to the literature the Mexican population has the highest T allele frequency of 0.59 and TT genotype frequency of 35% [12].The same study reports the lowest T allele frequency in sub-Saharan African and Canadian Inuit populations. Also, there are differences among the European populations. The frequency of heterozygous carriers is the largest in Italy (44%), as opposed to Norway, where it is lower (28%). Meta analysis of studies among European populations has shown that the frequency of homozygous carriers ranges from 5–15% [10,13]. Rosenberg et al showed that the *MTHFR 677T* variant has occurred on a common haplotype in Israelis, Japanese, and Ghanaians [14].

In our study the statistical analysis showed no significant difference in the prevalence of C677T *MTHFR* polymorphism between males and females carriers. The frequency of 44.44% heterozygous and 11.11% homozygous of C677T *MTHFR* polymorphism cases among representing Southern east Bosnian population is consistent with the data from literature. Most of the studies reviewed did not specify the gender composition of the samples, did not comment on differences in genotype frequencies by sex, or reported that genotype frequency was not significantly different in males and females [15].

Beside of high frequency of C677T polymorphism at global level we can speculate that it is evolutionary very old. The reason for high frequency of the T allele in many populations is unclear. Fodinger et al. postulated hypotheses that in time of great death, reduced activity of *MTHFR* which leads to decreased metilation of homocysteine and allowed C1 units of tehtrahydrofolate metabolism to be accessible for nucleotide synthesis [16]. On the other hand the benefit deriving from a high dietary intake of folate by *677T* allele carriers may be due to various mechanisms, such as the increased synthesis of purines and pyrimidines needed for DNA replication [17]. In addition, folate may neutralize the adverse cellular effects of the reduced activity of the

thermolabile *MTHFR* variant, such as the defective remethylation of homocysteine, the subsequent DNA hypomethylation, and the uracile misincorporation and it may also influence epigenetic mechanisms regulating gene expression [11].

The C677T transition in the *MTHFR* gene results in elevated levels of plasma homocysteine in homozygous carriers, especially in the presence of low folate levels [15]. The variant enzyme has reduced activity and inherited as an autosomal recessive trait. In many populations the inherited thermolabile variant for *MTHFR* is associated with higher serum homocysteine levels. This polymorphism has been regarded as a genetic risk factor for various disorders, such as coronary artery diseases, neural tube defects, cleft lip, venous thromboembolism, recurrent spontaneous abortions, primary angle glaucoma [18-24]. Also, the relationship between the *MTHFR* C677T polymorphism and its exact action with type 2 diabetes lellitus (T2DM) worldwide is under question. Several previous studies have shown no association between the *MTHFR* C677T polymorphism and T2DM among Taiwanese, Tunisian, Brazilian, German and Czech populations. In contrast, other data have indicated a significant association with T2DM among Moroccan, Chinese and Polish populations [25].

## Conclusion

This study is the first to report frequency of C677T *MTHFR* polymorphism in healthy Bosnian population. We did not find statistically significant difference in frequency of C677T *MTHFR* polymorphism among males and females subject in representing Southern east Bosnian population. Frequency of the T allele and C677T *MTHFR* genotype observed in this study is consistent with the data from literature.

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