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# METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T MUTATION IN TURKISH PATIENTS WITH THROMBOSIS*

*Günay Balta PhD**, Aytemiz Gürgey MD****

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**Recently, the homozygote state for the thermolabile variant of the MTHFR gene (C677T) has been identified as a determinant of elevated homocysteine levels which are known to be a risk factor for arterial and thrombotic vascular disease. To determine whether this variant increases the risk of thrombosis, we analyzed the prevalence of the C677T substitution in the MTHFR gene in 94 patients with thrombosis and in 95 unmatched controls. Although homozygosiy for he mutation was found in 12 (12.8%) of the patients with thrombosis and in only six (6.3%) of the control subjects, the difference in the prevalence of the homozygous mutant genotype between patients and healthy subjects was not statistically significant.** *Key words: methylenetetrahydrofolate reductase, MTHFR, C677T mutation, thrombosis, hyperhomocysteinemia, thermolabile enzyme.*

Elevated plasma levels of homocysteine appear to be a risk factor for arterial disease and for venous thrombosis. One of the metabolic pathways for homocysteine involves the enzyme methylenetetrahydrofolate reductase (MTHFR), which is responsible for the conversion of homocysteine to methionine. Frosst et al[1]. recently reported a C to T substitution at nucleotide 677 of the MTHFR gene that converts an alanine to a valine residue. Later, it was reported that homozygosity for the mutation was associated with a three-fold increase in the risk for premature cardiovascular disease[2]. Individuals homozygous for the thermolabile variant of the MTHFR gene due to this substitution have significantly elevated plasma homocysteine levels which may account for one of the genetic risk factors of arterial disease[1]. However, the risk for thrombotic vascular disease remains controversial. We have, therefore, evaluated the importance of the C6677T mutation in Turkish patients with thrombosis.

## Material and Methods

Analysis of the MTHFR 677 C-T substitution was performed as previously described[1]. Genomic DNA was isolated from peripheral blood by standard methods[3]. A fragment of the MTHFR gene was amplified by polymerase chain

* From the Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Molecular Biologist, Hacettepe University Faculty of Medicine.

*** Professor of Pediatrics and Pediatric Hematologist, Hacettepe University Faculty of Medicine.

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reaction (PCR) as described[1]. Polymerase chain reaction products, 198 bp in size, were digested with restriction endonuclease Hinf I which recognizes C to T substitution at the nucleotide 677. The presence of 198 bp (normal) and 175 bp (mutant) DNA fragments was observed in a two percent agarose gel (Fig. 1). Statistical analysis was carried out by chi-square testing.

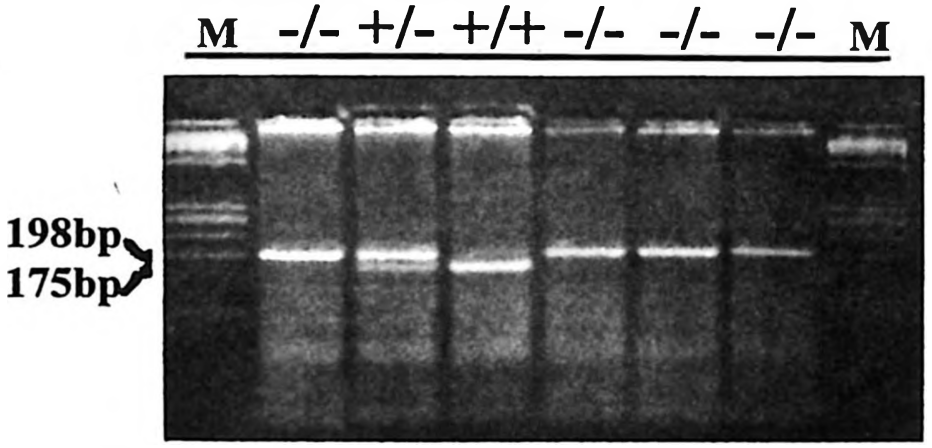


Fig. 1: Analysis of the C to T substitution at nucleotide 677 of the MTHFR gene by Hinf I digestion. The substitution creates a Hinf I recognition sequence which digests the 198 bp fragment into 175 and 23 bp fragments; the latter fragment has run off the gel. Heterozygous (+/-) and homozygous for mutated allele (+/+) or normal allele (-/-) patterns are shown in the figure. M: ϕX174/Bsu RI (Hae III) Marker.

## Results

Among 94 patients with thrombosis, 12 (12.8%) were homozygous and 33 (35.1%) were heterozygous for the C677T mutation. In 95 controls, six subjects were homozygous (6.3%) and 50 subjects (42%) were heterozygous (Table I). Although the frequency of the mutation in thrombotic patients was twice that as in the control group, no significant difference for homozygosity was detected between thrombophilic and control subjects by chi-square testing (p = 013).

Table I: Distribution of Genotypes and Alleles of MTHFR C677T Substitution

| | | Genotype | | | Alleles | |
|---|---|---|---|---|---|---|
| Group | Number | -/- | -/+ | +/+ | - | + |
| Thrombotic patients | 94 | 49 (52%) | 33 (35.1%) | 12 (12.8%) | 131 (69.7%) | 57 (30.3%) |
| Control subjects | 95 | 49 (52%) | 40 (42%) | 6 (6.3%) | 138 (72.6%) | 52 (27.4%) |

## Discussion

Hyperhomocysteinemia is known to be associated with an increased risk of both arterial and venous thromboembolic disease[1,2]. Previous reports concerning the C677T mutation in the MTHFR gene suggest that this variant may be associated with an increased risk for coronary artery disease[1]. The prevalence of

homozygotes for the C6677T mutation may vary significantly in populations from different geographic areas (1.4% to 29.7%)[4,5]. However, its role in the pathogenesis of venous thrombosis remains controversial. Some authors reported that the mutation is higher in thrombotic patients than in the healthy control population[1-2]. Others have suggested that the MTHFR C677T mutation is not associated with an increased risk of thrombosis[6,7]. Recently, the occurrence of an interaction between the MTHFR genotype and folate status was shown, and when plasma folate concentrations were below the median, plasma homocysteine levels were significantly higher in homozygotes for the C677T mutation than in those with the normal genotype[8].

In the present study, although there was quite a difference the frequency of homozygosity for the C677T mutation in patients with thrombosis and in healthy controls, no difference in the prevalence of homozygosity for the mutation was found in patients and control subjects by statistical tests. Therefore, our data suggest that the MTHFR C667T mutation was not associated with an increased risk of thrombosis in our population. However, further studies in a larger number of patients are needed to determine whether it is an added risk factor in individuals with other thrombotic abnormalities.

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