



Methylenetetrahydrofolate Reductase (MTHFR) Gene C677T Polymorphism Is Associated with Coronary Atherosclerosis Disease in a Sample of Iranian Patients

Ahmad Aleyasin, PhD^{1*}, Mahboobeh Ghaedi¹, Saeed Davoodi, MD², Seyed Hesameddin Abassi, MD², Manouchehr Madani, MD²

¹National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.

²Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran.

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Abstract

Background: Several studies showed that elevated plasma homocysteine level is a risk factor for coronary artery disease (CAD). A common polymorphism C677T of methylenetetrahydrofolate reductase (MTHFR) gene is reported to be associated with decreased enzyme activity and increased blood homocysteine level.

Methods: This study evaluated the association between C677T polymorphism and blood homocysteine level with CAD in 100 patients compared to 100 normal controls.

Results: Higher prevalence of the C677T polymorphism as well as elevated level in blood homocysteine were observed in Iranian CAD cases compared to the normal control. The C677T MTHFR common polymorphism was significantly associated with CAD, supported by a P value 0.032 and Chi-square equal to 6.87.

Conclusions: The TT genotype of MTHFR gene was attributed to increased blood homocysteine level in patients compared to T/C and C/C genotypes in studied Iranian cases. This study shows the advantage of testing C677T polymorphism in affected patients as a risk factor for coronary artery disease.

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Introduction

Homocysteine is a thiol-containing amino acid produced from methionine metabolism. Elevated plasma homocysteine is generally accepted as an independent risk factor for cardiovascular disease and venous thrombosis.¹⁻⁵ Enhanced risk associated with a 5 μmol/l elevated total homocysteine was estimated to be the same as that associated with a 0.5 μmol/l increased total cholesterol in CAD patients.⁶ Homocysteine is involved in two main metabolic pathways; transsulfuration in which homocysteine is catalyzed to cysteine by cystathionine β synthase and Vit B6 and remethylation in

which homocysteine is converted to methionine in a reaction catalyzed by methionine synthase. The methyl donor in the second reaction is methylenetetrahydrofolate (MTHF) which is converted by methylenetetrahydrofolate reductase enzyme (MTHFR).⁷

Common polymorphism of C to T substitution at the nucleotide position 677 of MTHFR gene causes alanine to valine substitution and produces thermolably sensitive form of enzyme. This polymorphism reduces enzyme activity and increases thermolability in lymphocyte extract. It is

*Corresponding Author: Ahmad Aleyasin, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran. P. O. Box: 14155-6343. Tel: +98-21-44580383. Fax: +98-21-44580395. E-mail: ahmad.aleyasin@nrcjeb.ac.ir.

associated with high plasma homocysteine level and has been involved in development of early atherosclerotic and thrombotic vascular diseases.⁸⁻¹³ Thermolabile MTHFR however accounts for mild hyperhomocysteinemia in approximately 25% of patients with vascular disease.^{7,14,15} The aim of this study was to assess the prevalence of the C677T MTHFR polymorphism and its association with plasma homocysteine level among CAD patients diagnosed in Tehran Heart Center.

Methods

Sampling

This study was carried out on 100 reetnelovpatients aged 32 to 56, who had angiography in Tehran Heart Center with angiographically documented CAD, at least 50% coronaryartery stenosis (left anterior descending, circumflex or right coronary artery) or a history of coronary angioplasty or surgical revascularization. A cardiologist consulted all chosen families. A control group consists of 100 randomly chosen normal volunteers, aged 32 to 55, without a history of CAD. Informed consent was obtained from all participants and the study was approved by the ethics committee of the center. Five milliliter of blood sample was obtained from cases and controls. Total fasting plasma homocysteine was measured for all patients and 100 controls using homocysteine measuring kit (Axis homocysteine enzyme immunoassay, Germany). For DNA analysis, standard salting out DNA extraction procedure was used to extract DNA from 200 collected blood samples for as previously described in Miller et al 1988.¹⁶

Polymorphism analysis of MTHFR gene

Hundred CAD patients and 100 healthy normal controls were tested for common C677T MTHFR polymorphism. A Pair of primers were designed to amplify a 254 bp fragment of MTHFR gene containing codon 677 forward and reverse primers were (5'GCC TCT CCT GAC TGT CAT CC3') and (5'GGA GCT TAT GGG CTC TCC TG3') respectively. PCR thermal cycle was performed in 32 cycles. Each cycle consisted of 95°C denaturation for 30 seconds, 60°C annealing for 1 minute and 72°C extension for 30 seconds. The thermal cycles began with an initial denaturation of 95°C for 5 minutes followed by a final extension of 72°C for 10 minutes. PCR product was exposed to restriction enzyme digestion with *HinfI* (Roche, Germany). The presence of the C677T Polymorphism within the MTHFR gene creates a *HinfI* restriction site that is detected by appearance of a 147 and 108 base pair fragments on a 10% polyacrylamide PAGE gel electrophoresis, visualized with silver staining (Figure 1).¹⁷

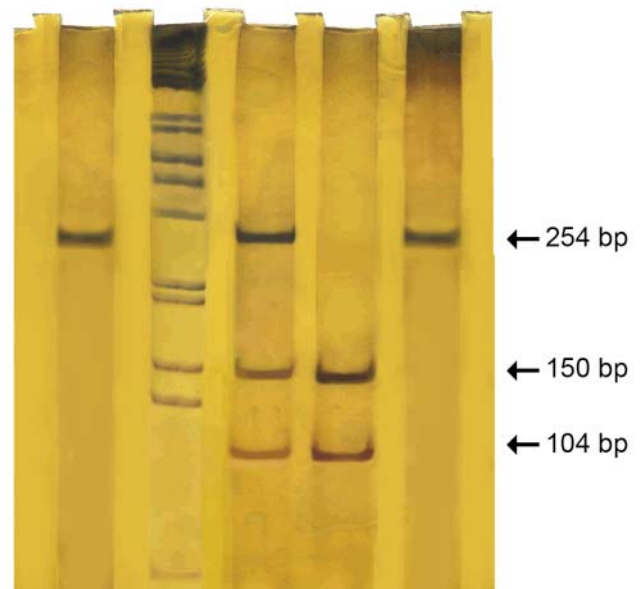


Figure 1. PCR product of MTHFR C677T fragment digested with *HinfI* restriction enzyme. From left to right line1, PCR product 254bp; line2, size marker ladder X: line 3, heterozygous C/T genotype with three cut and uncut fragments; line 4, homozygous T/T genotype with two cut fragments, line 4, homozygous C/C genotype with no cut fragment.

Statistical analysis

The predicted number of subjects required for meaningful analysis was determined based on the following assumptions: the predicted mean \pm standard deviation homocysteine plasma concentration in healthy subjects (10.5 \pm 2.8 μ mol/L), difference in the homocysteine level between patients and controls (10%) and the level of significance 0.05. Allele frequencies were calculated for each genotype by allele counting. Descriptive values were expressed as the mean \pm SD. Comparisons of allele frequencies between case and control groups were determined using a Pearson χ^2 test using SPSS for windows version 9.0 (Chicago, Illinois) software. Differences between patient and control group were assessed by student t test for continuous variables (Homocysteine). Fisher exact test was used when the number of observation in any group was less than or equal to 5. All tests were two-tailed and $p < 0.05$ was considered as significant.

Results

Hundred CAD cases and 100 normal controls were genotyped for common polymorphism C677T MTHFR and blood homocysteine level to determine their association with CAD disease. The mean age of case and control was 48.2 and 47.8 respectively, from whom 30% were female. The frequency of mutated C677T MTHFR polymorphism



was 33.5% among 100 cases and 22% among 100 normal controls (Table 1).

Table 1. Distribution of C677T polymorphism of the MTHFR gene and its genotype frequencies among CAD cases and normal controls

Groups	Normal (C/C)	Heterozygote (T/C)	Mutant (T/T)
Case	43.0 (34.0%)	47.0 (47.0%)	10.0 (10.0%)
Control	61.0 (61%)	34.0 (34.0%)	5.0 (5.0%)

The frequencies of the C/C, C/T, and T/T genotypes among control group were 61.0%, 34.0% and 5.0% respectively whereas the corresponding frequencies among the patients with CAD syndrome were 43%, 47% and 10% respectively. The incidence of the thermolabile C677T MTHFR in 100 patients with CAD and 100 controls was compared (Table 1). The difference between the two groups was significant ($\chi^2 = 6.87$ and $P=0.032$). There was no significant difference in the level of thermolabile MTHFR among male and female in either patient ($\chi^2 = 1.32$, $P=0.420$) or control ($\chi^2 = 4.9$, $P=0.08$), as well as for age in either patient ($\chi^2 = 3.6$, $P=0.162$) or control ($\chi^2 = 5.9$, $P=0.380$) groups.

The level of plasma homocysteine was compared among patients and controls. The proportion of homocysteine was substantially higher among patients with CAD than that among the controls. The difference of blood homocystein level between two groups was significant 14.93 ± 5.23 versus 11.81 ± 5.75 and $P < 0.05$. The correlation between MTHFR genotypes T/T, C/T and C/C and homocysteine level in cases was 18.75, 15.32 and 14.93 in comparison to 13.68, 11.07 and 11.81 for controls, respectively (Table 2). The T/T genotype was associated with high levels of plasma homocysteine when compared to C/T and C/C genotypes.

Table 2. Distribution of plasma homocysteine concentration ($\mu\text{mol/l}$) in different MTHFR genotypes among CAD cases and normal controls.*

Groups	Normal (C/C)	Heterozygote (T/C)	Mutant (T/T)
Case	14.93 ± 5.23	15.32 ± 6.36	18.75 ± 7.86
Control	11.81 ± 5.75	11.07 ± 5.42	13.68 ± 5.70

* Data are stated as mean \pm SD

Discussion

The association between C677T MTHFR genotype and CAD were studied in 100 Iranian CAD cases and 100 normal controls. The frequency of mutated T allele in patients was higher than that in the normal control group supported with a significant P value equal to 0.032. This study showed a

significantly higher prevalence and a positive relationship of C677T polymorphism MTHFR gene among CAD in Iranian cases. Our study is consistent with the hypothesis that C677T MTHFR polymorphism may be an important risk factor for CAD in a selected population. The frequency for C677T heterozygosity of MTHFR gene in normal control is the same as that reported for Caucasian subjects. The frequency of heterozygosity (34.0%) in this study is consistent with the previous reports for heterozygosity (34-59%) of various populations.¹⁸ The heterogeneous distribution of the C677T polymorphism of MTHFR gene in the cases may partly be explained by the varying relationship of heart disease risk through decreased MTHFR activity.

The level of plasma homocysteine was also measured among patients and normal controls. The level of homocysteine is age related and increases in older persons. The mean plasma homocysteine in T/T genotype was 18.75 ± 7.86 compared to 13.68 ± 5.70 for the control group ($P < 0.05$).

Individuals homozygous for T/T genotype were associated with higher levels of plasma homocysteine level than C/T and C/C genotypes. There was a small increase in homocysteine levels in patients with C/T genotype. This data is in line with several previous studies that have found elevated plasma homocysteine concentration in CAD patients with T/T genotypes. It is in accordance with previous studies that suggest folate abnormalities appear to play a major role in the pathogenesis of increased homocysteine level in older persons.¹⁹

Numerous studies have demonstrated a significant relationship among C677T polymorphism, homocysteine concentration and CAD, however some reports have suggested no relationship between risk of CAD and C677T polymorphism.²⁰⁻²⁸ Kluijtmans and Whitehead²⁹ performed a meta-analysis of the first 10 studies that argued an increase risk of CAD in patients with TT genotype. They demonstrated a 30% increased risk of CAD associated with TT and CT genotypes (OR 1.27, 95% CI 1.11-1.44).

In a report from the Polish population,³⁰ which included 100 patients after MI and 100 healthy volunteers, no significant relationship was shown between MTHFR genotype and age where the MI happened. However, different results were obtained in the Turkish population where 96 males who suffered MI below the age of 45 with TT genotype showed almost six-fold higher risk of MI than other genotypes found in 100 healthy volunteers who had 5% incidence of TT genotype.³¹

On the other hand, some workers who found no relationship between polymorphism and CAD had studied older patients.^{24,25,30} Payne and colleagues³² have suggested an obscuring of the correlation of the polymorphism with premature onset of CAD in selected patients that achieved a negative relationship. Our study highlights the potential prognostic significance of the C677T substitution in MTHFR gene in patients with coronary artery disease in a sample of Iranian patients.

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