Research in Molecular Medicine

Original Article
2014, Vol: 2, Issue: 3, Pages: 36-40
DOI: 10.18869/acadpub.rmm.2.3.36

Association of Methylenetetrahydrofolate Reductase (*MTHFR*) Gene C677T and A1298C Polymorphisms with Myocardial Infarction from North of Fars Province

Mahboobeh Nasiri [1*], Ali Roostaei [2], Zeinab Ehsanian [1]

[1] Department of Natural Sciences, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran.
[2] Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran.

Received: 20 Feb 2014

Revised : 10 Apr 2014

Accepted: 25 Apr 2014

Corresponding Authors:
Mahboobeh Nasiri
Department of Natural Sciences, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran.
Phone: +98-917-3010601
E-mail: nasiri@iaua.ac.ir

Abstract

Background: The association between Methylene tetrahydrofolate reductase polymorphism and Coronary Artery diseases risk has been both confirmed and refuted in a number of published studies. The aim of this study was to investigate whether genetic polymorphisms of *MTHFR* (C677T, A1298C) contributed to the development of myocardial infarction (MI).

Material and Methods: The present case-control study consisted of 54 patients with a history of MI and 54 genders matched normal controls. The SNPs genotypes were determined using polymerase chain reaction followed by restriction fragment length polymorphism method.

Results: No significant association of the *MTHFR* A1298C with the risk of MI was observed. However, the allele frequencies of C677T SNP differed significantly among patients and controls (0.83 vs. 0.30). A strong positive relationship between the TT genotype and the risk of MI supported with a significant P value < 0.001 (OR= 11.87, 95% CI: 4.7- 29.9, p < 0.001).

Conclusions: The results of present study show the importance of C677T SNP as a potential biomarker for screening susceptible cases to MI.

Keywords: Methylenetetrahydrofolate Reductase; Myocardial Infarction; Polymorphism; Iran

Please cite this article as: Nasiri M, Roostaei A, Ehsanian Z. Association of Methylenetetrahydrofolate Reductase (MTHFR) Gene C677T and A1298C Polymorphisms with Myocardial Infarction from North of Fars Province. Res Mol Med. 2014; 2 (3): 36-40

Introduction

Coronary artery disease (CAD) is the common cause of death in developed countries and its prevalence is rising rapidly in developing countries (1). Myocardial infarction (MI) is the most acute form of CAD (2). CADsareclassifiedascomplexmultifactorial disorders, which involved the contributions of many different genes, as well as environmental factors. A major current challenge is therefore to elucidate the genetic components that contribute to the pathogenesis of complex diseases (3). Several recent studies accumulate data in favor of the Homocysteine metabolism down regulation as a risk factor for congenital heart diseases (CHDs), MI and stroke (4-6).Homocysteine is a sulfur amino acid normally found in blood. Elevated homocysteine levels (also called hyperhomocysteinemia) may cause irritation of the blood vessels.Upper levels of homocysteine show an increased risk for hardening of the arteries (atherosclerosis), which could eventually result in a heart attack and/ or stroke, and blood clots in the vein, referred to as Venus thrombosis (3,7). Hyperhomocysteinemia can result from genetic factor like mutation in Methylenetetrahydrofolate reductase (*MTHFR*) gene or due to environmental factor like deficiency of B12 or folic acid (4). MTHFR is a vitamin B12- dependent enzyme, plays a crucial role in remethylation of homocysteine to methionine by 5-methyltetrahydrofolate which coded by the gene on chromosome 1 (1p36.3). Any changes in *MTHFR* gene may disrupt this conserved regulation pathway, which lead to hyperhomocysteinemia (8, 9).

There are two common polymorphisms in the gene codes for the MTHFR enzyme, C677T and A1298C. The codon 677 polymorphism (rs1801133), in exon 4

of the gene, lies within the NH_2-terminal catalytic domain, whereas the codon 1298 polymorphism is in the COOH-terminal regulatory region in exon 7 (3). Individuals who have a C to T substitution at base 677 of the gene (amino acid change A222V) have reduced enzyme activity and higher homocysteine (3, 10, and 11). The second prevalent polymorphism A1298C (rs1801131) is also associated with decreased enzyme activity in vitro. This genetic variant consists of an A to C transversion at nucleotide 1298, which producesa Glu to Ala substitution (10).

Elucidation of an association, if any, between these polymorphisms and MI risk might be informative regarding the hypothesis that impaired folate metabolism, resulting in high homocysteine concentrations, plays a causal role in the occurrence of MI. The aim of the present study was to assess the prevalence of SNPs of *MTHFR* gene (C677T and A1298C) and its relation to MI by conducting a case - control study.

Materials and Methods

Subjects

A total 108 subjects, 54 patients (mean age 51.4 ± 13.5) with at least one experience of MI and 54 controls (mean age 51.6 ± 13.2) with no previous history of heart diseases were enrolled in this study from Marvdasht town in Fars province. Group matching of cases and controls was done for age and sex. All participants gave their informed consent prior to their inclusion in the study.

Molecular analysis

Genomic DNA was extracted from whole blood by standard phenol- chloroform salting out method. The quality of extracted gDNA was checked on 1.5% agarose gel.

Genotypic analysis for both SNPs (C677 & A1298) were determined in DNA by PCR-RFLP assay. Two pair of primers was designed to amplify the flanking regions of codon 677 and 1298 in *MTHFR* gene which resulted in 198bp and 143bp amplicons respectively (Table 1).

PCR analysis of both 677 and 1298 gene polymorphisms were carried out in a total volume of 25 ml, containing genomic DNA (50ng); 1.5 mM $MgCl_2$, 200 mM dNTP, 10 pmol/µl of each primer; 1x Taq polymerase buffer and 1 unit of Taq DNA Polymerase (Cinnagen, Iran).

Table 1. Primer sequence and restriction endonuclease used for genotyping

Primer name	Sequence 5′→3′	Product size (bp)	Restriction enzyme
MTHFR 677 F	TGAAGGAGAAGGTGTCTGCGGGA	198	*Hinf* I
MTHFR 677 R	AGGACGGTGCGGTGAGAGTG		
MTHFR 1298 F	GCAAGTCCCCCAAGGAGG	143	*Mbo* II
MTHFR 1298 R	GGTCCCCACTCCAGCATC		

For C677T, the thermocycling procedure consists of initial denaturation at 94 ºC for 3 min, followed by 30 cycles of denaturation (94 ºC for 1 min), annealing (67 ºC for 1 min) and extension (72 ºC for 1 min), and final extension (72 ºC for 7 min). Amplification was achieved with a gradient BioRad t100 thermocycler (USA). Amplicons were resolved as a single band by 3% agarose gel electrophoresis prior to RFLP analysis to ensure that a specific single product was amplified. The PCR product was incubated with 1 unit of *Hinf* I (MBI Fermentas) at 37 ºC for 3 hours, using the buffer supplied by the manufacturer.

The digested product was separated on 3% agarose gel electrophoresis and photographed using Uvitec (Cambridge, UK) gel documentation system. After electrophoresis, the gel was stained with ethidium bromide. The sizes were determined using 100 bp ladder (Fermentas, Germany). The restriction site resulted from C to T transition, is located at the GTC codon encoding the amino acid valin forming a cuttable site. An uncuttable site at codon GCC encoding the amino acid alanin will remain intact (198 bp equal to wild type homozygous TT). If the product is excisable; two fragments, 175 and 23 bp, will be present (polymorphic CC homozygous).

Heterozygous genotype is known in the presence of 198, 175 and 23bp fragments. In the case of *MTHFR* 1298, PCR was done with initial denaturation of 95 ºC for 1 min, followed by 30 cycles of 95 ºC for 30 sec, 61 ºC for 30 sec, and 72 ºC for 30 sec, and a final extension at 72 ºC for 7 min. Ancestral allele at this position refers to as “A” allele, locates at the second position of the GAG codon for glutamic acid. A to C transition emerge the GCG codon for Alanine and the new cutting site for *Mbo*II restriction enzyme (Fermentas, Germany). As a result of digestion, AA wild type genotype produces three fragments of 38, 28 and 77bp whereas; polymorphic homozygous CC identified by two fragments length 38 and 105bp fragments.

Statistical analysis

A $\chi 2$ test was performed for both C677 and A 1298

polymorphism to determine if the samples demonstrate Hardy- Weinberg equilibrium. Unconditional logistic regression was used to calculate ORs and 95% CI for MI risk associated with the genetic polymorphisms of MTHFR gene. In these analysis the reference group consisted of putative low risk genotypes (CC genotype of C677 and AA genotype of A1298). Statistical analysis were carried out using SPSS 16.0 for window software (SPSS, Inc., Chicago, IL, USA). A p value of less than 0.05 was considered statically significant.

Results

The average age of the patients were 51.4 ± 13.5 (range from 24 to 81), as well as in control group with mean age 51.6± 13.2 (range 24 to 81). The results of PCR amplification yielded two amplicons, length 198bp and 143bp For C677T and A1298C (Figure 1 and Figure 2).

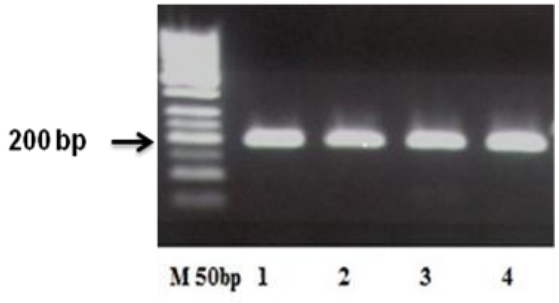

Figure 1. The PCR fragment obtained from the designed primers to flanking sequence of the SNP is 198bp in length. (M: marker, 50 bp ladder, Fermentas, Germany)

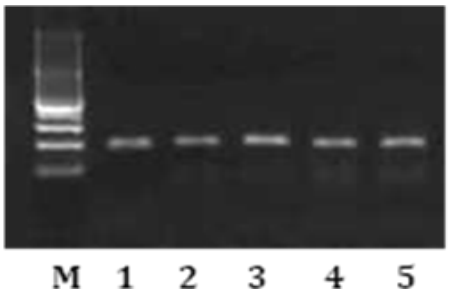

Figure 2. The PCR fragment amplified by specific pair of primer designed for MTHFR1298 polymorphic site and its flanking region yielded a 143 bp amplicon. (M: marker, 50 bp ladder, Fermentas, Germany)

Post digestion documentation was done on gel and as shown in figures 3 and 4, TT, CC (for C677T) and AA, AC, CC (for A1298C) genotypes were seen (Figure 3 and Figure 4).

Detailed genotypic distributions for studied SNPs were summarized in Table 2. Control subjects for the genotypic frequencies of A1298 polymorphism were in Hardy- Weinberg equilibrium (χ^2= 4.8, df= 2, p= 5.99), but not for C677 (χ^2= 41.34, df= 1, p= 3.84). Logistic regression analysis showed that there was no significant association between A1298C polymorphism and susceptibility to MI (Table 2).

As statistical analyses on C677T showed, the polymorphic variants were more prevalent among patients, whereas the wild types were more commonly seen in controls. The frequencies of the CC and TT genotypes among control group were 70.4% and 29.6% respectively,whereas the corresponding frequencies in MI patient group were 16.7% and 83.3%, respectively. The homozygous polymorphic genotype TT showed statistically strong significant association with MI (OR= 11.87, 95% CI: 4.7- 29.9, p < 0.001, Table 2).

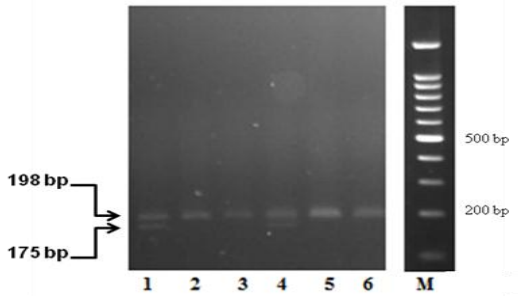

Figure 3. PCR- base restriction analysis of *MTHFR* C677T polymorphism shown on 3% agarose electrophoresis. The polymorphic region was amplified by PCR resulting in a digestible fragment in lane 1 and 4 (TT homozygote), an indigestible fragment in lane 2, 3, 5, 6 (TT homozygote). M: marker (right side), 100 bp ladder

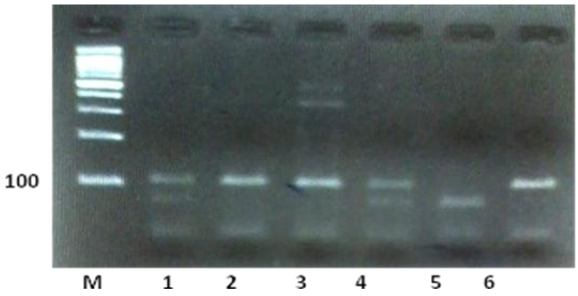

Figure 4. PCR-base restriction analysis of *MTHFR* A1298C polymorphism shown on 3% agarose electrophoresis. The polymorphic region was amplified by PCR resulting in digestible fragment length 105 and 28 bp in lane 2, 3, 6 (CC homozygote), other digestible fragment length 77 and 28 bp in lane 5 (AA homozygote). Three different fragments 105, 77, 28 bp represent AC heterozygote. M: marker, 100 bp ladder (Fermentas, Germany).

Nobody with CT genotype were found in both patient and control groups. Allele frequencies for C677T SNP among patients and controls were 0.17 and 0.7% for allele C (wild type), respectively and 0.83 and 0.3% for allele T (mutant), respectively (Table 3). As the statistical results showed, compared with controls, there was a higher T allele frequency of C677T polymorphism in cases, 0.83 vs. 0.3.

Discussion

Polymorphisms in MTHFR gene, which involved in metabolism of homocysteine, were analyzed including MTHFR C677T and MTHFR A1298C to explore the genetic susceptibility to homocysteine toxicity and risk of MI. In the present study, all genotype frequencies for A1298C SNP were in Hardy - Weinberg equilibrium. The frequency of C allele was the same in cases and controls (0.47%). In our study, association found neither between CC (OR= 0.97, 95% CI: 0.3-3.15, p= 0.96) nor AC (OR= 1.96, 95% CI: 0.78- 4.91, p= 0.15) with the risk of MI in cases compared to the normal controls. This could be because of the reduced sample size. But there are reports on the importance of this polymorphism in the etiology of CHDs (6).

Table 2. Association between MTHFR 677 and 1298 polymorphisms and MI risk.

Polymorphism	Genotype	Patients n (%)	Controls n (%)	Odd ratio (95% CI)	P-value
C677T	CC	9 (16.7)	38 (70.4)	Reference	
	TT	45 (83.3)	16 (29.6)	11.87 (4.7- 29.9)	<0.001
	Total	54 (100)	54 (100)		
*A1298C	AA	11 (20.2)	16 (29.6)	Reference	
	AC	35 (64.9)	26 (48.2)	1.96 (0.78-4.91)	0.15
	CC	8 (14.9)	12 (22.2)	0.97 (0.3-3.15)	0.96
	Total	54 (1000	54 (100)		

For *MTHFR* C677T, the polymorphic homozygous TT was the most prevalent genotype found in study population (83.3%), followed by wild type homozygous (CC) (19.6%). Heterozygosity for C677T was not evaluated in both controls and patients. The T allele in the present study was found to be associated with higher MI risk (0.83 in patients vs. 0.30 in normal controls). The frequency of occurrence of the polymorphic allele has been shown to vary among different ethnic groups of the world (4, 12). The result of our allelic frequency is in consistent with the previous research on the association of C677T with CAD in Iranian sample population (13). In a report from Egyptian population, which included 40 cases and 25 controls, no significant association was shown between T allele and CAD (P>0.05) (14). Our study is also at variance with a study from Pandey *et al* (2011), which found no significant association between T allele and CAD in Indian patients (C vs. T; OR= 1.24, 95% CI: 0.84-1.85, p= 0.25) (3). The strong relationship was shown for the *MTHFR*677 T allele and the risk of stroke happening (OR= 22.29, 95% CI: 4.89-98.8) (15).

Table 3. Allele frequencies of MTFHR C677T and A1298C among patients and controls

MTHFR	Allele	Patients (n=54)	Controls (n=54)
677 C/T	C	0.17	0.7
	T	0.83	0.3
1298 A/C	A	0.6	0.65
	C	0.4	0.35

MTHFR gene is involved in the remethylation of homocysteine to methionine. The activity of *MTHFR* enzyme is a crucial factor determining the level of homocysteine in plasma (9). Two common polymorphisms reports for this gene. C677T exhibit a C to T transition at codon 222, which substitutes conserved Val with Ala. This replacement reduces the activity of the enzyme (16). A1298C results from an A to C transversion which decreased the enzyme activity measured *in vitro* (17). Therefore, the polymorphisms may affect the homocysteine, endothelial toxin, which introduces as a risk factor for atherosclerotic disease including stroke and MI.

Statistically significant correlation was found in our study between TT genotype and the risk of MI (OR= 11.87, 95% CI: 4.7-29.9, p<0.001). Our study is consistent with the hypothesis that C677T *MTHFR* polymorphism may be an important risk factor for the MI in a selected population (13).

Kluijtmans and Whitehead performed a meta-analysis of the first 10 studies that argued an increased risk of CAD in patients with TT genotype. They demonstrated 30% increased risk of CAD associated with TT and CT genotypes (OR = 1.27, 95% CI: 1.11- 1.44) (18). The result of a meta-analysis carried out by Klerk *et al* (2002) demonstrated that individual with the *MTHFR* 677 TT genotype has a 16% higher odds of CHD compared with individuals with the CC genotype (5). A recent meta analysis by Clarke et al (2012) studied 19 unpublished data sets and found only 2 percent increase risk of CAD in TT homozygotes as compare to CC homozygotes (19), in contrast the meta analysis of 86 published studies showed of the association of the polymorphism with CAD, the excess CAD risk in TT homozygotes as compared to CC was 15 percent. The reason for this considerable discrepancy could be due to publication bias (19).

Numerous studies have demonstrated a significant relationship among C677T polymorphism and CAD, however some reports have suggested no relationship

between risk of CAD and C677T polymorphism. A recent meta-analysis of 80 studies has given an estimate of a 14% (95%CI: 5-24) greater risk of CAD associated with the MTHFR CC genotype (20). A meta-analysis of MTHFR C677T polymorphism and CAD found no strong evidence to support the association of this polymorphism with CAD in Europe, North America or Australia but in Asia (21). This geographical variability may be attributed to higher folate intake in these countries.

The results suggested that *MTHFR* C677T polymorphism is an informative genetic variant which can be used as a diagnostic marker for predisposed individuals to MI. The association between *MTHFR* C677T variant and CADs is obvious but, further studies are necessary to ascertain the relationship between the TT genotype of *MTHFR* 677 and homocysteine levels in plasma to clear the mechanism of its acting in increasing the risk of atherosclerosis.

Acknowledgements

The authors are indebted to the participants for their close cooperation. The authors have no conflict of interest in relation to this study.

References

1. Tardif JC. Coronary artery disease in 2010. Eur Heart J. 2010; 12:2-10.

2. Um JY, Kim HM. Tumor necrosis factor alpha gene polymorphism is associated with cerebral infarction. Brain Res Mol Brain Res. 2004; 122(1):99-102. PMID: 14992821

3- Pandey U, Kumari R, Nath B, Ganesh S, Banerjee I, Hasan O. Association of angiotensin- converting enzyme, Methylene tetrahydrofolate reductase and paraoxonase gene polymorphism and coronary artery disease in an Indian population. Cardiol J. 2011; 18(4):385-94. PMID: 21769819

4. Eftychiou C, Antoniades L, Makri L, Koumas L, Costeas PA, Kyriakou E, et al. Homocysteine Levels and MTHFR Polymorphisms in Young Patients with Acute Myocardial Infarction: A Case Control Study. Hellenic J Cardiol. 2012; 53(3):189-94. PMID: 22653243

5. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677 C to T polymorphism and risk of coronary heart disease. JAMA. 2002; 288(16):2023-2031. PMID: 12387655

6. Wang W, Wang Y, Gong F, Zhu W, Fu S. MTHFR C677T Polymorphism and Risk of Congenital Heart Defects: Evidence from 29 Case-Control and TDT Studies. Plos one.2013; 8(3):e58041. PMID: 23536781

7. Deeparani T, Pillai MR, Elavazhagan T. Detection of MTHFR C677T and A1298C gene polymorphism in congenital heart disease. MEJSR. 2009; 4(2):127-132.

8. Dhar S, Chatterjer S, Ray S, Dutta A, Sengupta B, Chakrabarti S. polymorphisms of the methylenetetrahydrofolate reductase gene as the genetic predispositions for coronary artery diseases in eastern India. J Cardiovasc Dis Res. 2010:1(3):152-7. PMID: 21187870

9. Gupta S, Kotwal J, Kotwal A, Dhall A, Garg S. Role of homocysteine & MTHFR C677T gene polymorphism as risk factors for coronary artery disease in young Indians. Indian J Med Res. 2012; 135(4):506-12. PMID: 22664498

10. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10(1):111-3. PMID: 7647779

11. Dedoussis GV1, Panagiotakos DB, Pitsavos C, Chrysohoou C, Skoumas J, Choumerianou D. An association between the methylenetetrahydrofolate reductase (MTHFR) C677T mutation and inflammation markers related to cardiovascular disease. Int J Cardiol. 2005; 100(3):409-14. PMID: 15837084

12. Yang B, Fan S, Zhi X, Li Y, Liu Y, Wang D, et al. Associations of MTHFR Gene Polymorphisms with Hypertension and Hypertension in Pregnancy: A Meta- Analysis from 114 Studies with 15411 Cases and 21970 Controls. PLoS ONE. 2014; 9(2):e87497. PMID: 24505291

13. Aleyasin A, Ghaedi M, Davoodi S, Abbasi SH, Madani M. Methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism is associated with coronary atherosclerosis disease in a sample of Iranian patients. J Tehran Heart Cent. 2006; 1(2):77-81.

14. Ibrahiem MM, Gaber O, Mohammed SH, Salem EM. Methylenetetrahydrofolate reductase C677T polymorphism and relationship with coronary artery disease. Egypt J Chem. 2009; 27(1):177-94.

15. Alluri RV, Komandur MS, Chawda K, Chaudhuri JR, Hasan Q. MTHFR C677T gene mutation as a risk factor for arterial stroke: a hospital based study. Eur J Neurol. 2005; 12:40-44.

16. Ezzat H, Attia FA, Mokhtar A, El-Tokhy HM, Alalfy MN, Elkhouly NY. Prevalence of thrombophilic gene polymorphisms (FVL G1691A and MTHFR C677T) in patients with myocardial infarction. Egypt J Med Hum Genet. 2014; 15:113-23.

17. Pazhakh V, Razmkhah F, Zaker F, Atashrazm F, Rasighaemi P. Methylenetetrahydrofolate Reductase Gene Polymorphisms and Risk of Myeloid Leukemia. LABMED. 2010; 41(8):490-4.

18. Kluijtmans L, Whitehead A. Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease. Eu Heart J. 2001; 22:294-9. PMID: 11161947

19. Clarke RDA, Parish S, Verhoef P, Dötsch-Klerk M, Lathrop M, Xu P. Homocysteine and coronary heart disease: Meta-analysis of MTHFR case-control studies, avoiding publication bias. Plos Med. 2012; 9(2):e1001177. PMID: 22363213

20. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677 C-T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005; 331:1053-8. PMID: 16216822

21. Cronin S, Furie KL, Kelly PJ. Does-related association of MTHFR 677T allele with risk of ischemic stroke: Evidence from a cumulative meta-analysis. Stroke. 2005; 36(7):1581-7. PMID: 15947278