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# Polymorphisms in methylenetetrahydrofolate reductase gene are not associated with acute myocardial infarction in a Pakistani population

*Mohammad Perwaiz Iqbal*, Saeed Mahmood, Siddiqa Parveen, Naseema Mehboobali and Ghulam Haider*
*Department of Biological & Biomedical Sciences, Aga Khan University, Stadium Road, Karachi, Pakistan*

**Abstract:** The objective of the study was to test the association of two polymorphisms of methylenetetrahydrofolate reductase gene, *MTHFR* C677T; *MTHFR* A1298C with acute myocardial infarction (AMI). A case-control study involving 308 AMI patients (age 30-74 years; 230 males and 78 females) and 319 age and gender matched normal healthy controls (235 males and 84 females) was carried out on a Pakistani population. Genotyping of the two polymorphisms was done using PCR-RFLP based assays. Fasting levels of plasma homocysteine and other biochemical parameters were determined using kit methods. Plasma homocysteine concentrations were found to be elevated in both cases and controls (18.1±7.7 µmol/l vs 18.1±8.1 µmol/l, respectively). Compared to Caucasian populations, homozygous variant genotype *MTHFR* 1298CC was found to be highly prevalent (27%) in Pakistani population. Neither *MTHFR* C677T nor *MTHFR* A1298C polymorphism was found to be associated with myocardial infarction (MI). Age-at-onset of MI was significantly affected by *MTHFR* C677T (TT=39 years vs CT/CC= 49 years; *P*=0.006). *MTHFR* polymorphisms appear to have no role in AMI in Pakistani population.

**Keywords:** Acute myocardial infarction, coronary heart disease, homocysteine, MTHFR C677T, MTHFR A1298C, polymorphism.
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***Author for Correspondence:** perwaiz.iqbal@aku.edu

## INTRODUCTION

Increased levels of plasma homocysteine have been reported to be associated with increased risk for the development of coronary heart disease (CHD)[1]. Methylenetetrahydrofolate reductase (MTHFR) is a folate and vitamin B12-dependent enzyme which is involved in the remethylation of homocysteine to methionine[2]. Two major polymorphisms in the gene for this enzyme (*MTHFR* C677T and *MTHFR* A1298C) have been indentified to have a role in hyperhomocysteinemia, thereby increasing the risk for cardiovascular disease[3,4]. While mutation in *MTHFR* C677T leads to increased thermolability of the enzyme, the mutation in *MTHFR* A1298C results into decreased activity of the enzyme[5]. In a previous communication we have reported that *MTHFR* C677T polymorphism, though associated with mild hyperhomocysteinemia, confers no significant risk of CHD in a Pakistani population[6]. Since South Asian populations have been shown to have high frequency of *MTHFR* 1298CC (variant form) genotype[7,8], it is important that its potential influence alone or in combination with *MTHFR* C677T genotypes on homocysteine levels and risk to CHD must be assessed on Pakistani population. A case-control study of dimorphisms, *MTHFR* C677T and *MTHFR* A1298C was carried out to investigate any association of these two dimorphisms with acute myocardial infarction (AMI, a presentation of CHD) and with plasma homocysteine in Pakistani subjects.

## MATERIALS AND METHODS

### *Patients and healthy controls*

Three hundred and eight consecutive Pakistani patients with AMI (age range: 30-74 years) admitted to the National Institute of Cardiovascular Disease, Karachi and Armed Forces Institute of Cardiology, Rawalpindi, were enrolled in this study with informed consent. Diagnosis of AMI was based on WHO criteria which included clinical history, ECG changes indicating myocardial damage and elevation of biochemical markers. These patients had first episode of myocardial infarction (MI). They were also assessed for other risk factors for CHD such as, diabetes mellitus (fasting serum glucose level>125 mg/dl); high blood pressure (systolic blood pressure>140 mmHg and diastolic blood pressure>90 mmHg); hypercholesterolemia (serum cholesterol level>200 mg/dl), hyperhomocysteinemia (plasma homocysteine level>15 µmol/l); hypertriglyceridemia (serum triglyceride (TAG) levels>200 mg/dl), obesity (a body mass index(BMI) greater than 27) and smoking (one or more cigarettes per day). Exclusion criteria used for these patients have been described in a previous publication[6].

Three hundred and nineteen apparently healthy subjects who had been matched for gender, socio-economic background and age (within 5 years) were enrolled with informed consent as controls. The study had been approved by the Ethics Review Committee of the Institution.

***Determination of biochemical parameters***

Fasting serum glucose, cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol were analyzed using kit methods (RANDOX, UK). Plasma homocysteine was determined using an immunoassay based kit (Abbott Laboratories, Ltd., Pakistan).

***Genotyping and data analysis***

Genomic DNA was extracted from the whole blood according to published protocols[9]. Genotyping of the two polymorphisms (*MTHFR* C677T and *MTHFR* A1298C) was done using polymerase chain reaction and resistriction fragment length polymorphism (PCR-RFLP) as per already published reports[3,10].

Data were analyzed using the SPSS® (Statistical Package for Social Sciences) Software Version 11.0 for Windows® (Gorinchem, The Netherlands). Distributions of polymorphisms in cases as compared to controls were assessed by using chi-square tests. Sub-phenotype associations were assessed by analysis of variance (ANOVA). Kaplan-Meier curves were constructed for MI age-at-onset (AAO) data. Estimations of departures from Hardy-Weinberg equilibrium, haplotype frequencies and association statistics for the polymorphisms were estimated using Haploview v3.2[11]. P-values less than 0.05 were considered statistically significant.

## RESULTS

The demographic and clinical characteristics of our patients and controls are described in Table 1. There was no difference in gender distribution but as a group, our controls were slightly younger than our MI patients, though both groups spanned a similar age range. The MI patients had higher BMI, and total serum cholesterol, LDL cholesterol and triglyceride levels.

**Table 1:** Demographic and clinical characteristics of AMI patients and control subjects.

| | MI (n=308) | Controls (n=319) | P-value |
|---|---|---|---|
| Age (years) | 51.4±11.1 | 46.1±11.1 | <0.001 |
| Age range (years) | 30-74 | 30-74 | |
| Gender (M/F) | 230/78 | 235/84 | 0.41 |
| BMI (Kg/m$^2$) | 25.2±3.9 | 23.8±4.9 | <0.001 |
| Total cholesterol (mg/dl) | 183.4±55.5 | 166.5±38.6 | <0.001 |
| LDL (mg/dl) | 109.4±48.8 | 97.1±33.9 | <0.001 |
| HDL (mg/dl) | 37.6±24.5 | 37.3±11.5 | 0.80 |
| Triglycerides (mg/dl) | 184.6±123.1 | 157.4±88.8 | 0.002 |
| Smoking (Y/N) | 93/214 | 48/257 | <0.001 |
| Glucose (mg/dl) | 136.51±78.5 | 95.4±33.3 | <0.001 |
| Homocysteine (μmol/l) | 18.1±7.7 | 18.1±8.1 | 0.97 |

The two functional single nucleotide polymorphisms (SNPs) were genotyped for *MTHFR*. Table 2 shows that *MTHFR* C677T polymorphism was in Hardy-Weinberg equilibrium in both the patient and control groups. *MTHFR* 1298CC genotype was found to be highly prevalent (27%) in both cases and controls. *MTHFR* 677TT genotype was in lesser proportion in AMI patients compared to normal healthy subjects (1.6% vs 2.8%, respectively). No genotype associations were found with any of the two *MTHFR* polymorphisms with MI (Table 2). Sliding window haplotype analysis also did not reveal any associations (Table 3).

**Table 2:** Allele frequencies, association and Hardy-Weinberg statistics for each of *MTHFR* polymorphisms

| No. | SNP | HWE | Genotypes (AMI; Controls) | $\chi^2$ (2-df) | P-value |
|---|---|---|---|---|---|
| 1 | A1298C | 0.01* | 47/173/81; 64/161/84 | 2.99 | 0.23 |
| 2 | C677T | 1.0 | 209/85/5; 228/74/9 | 2.50 | 0.29 |

SNP: Single nucleotide polymorphism, HWE: Hardy-Weinberg Equilibrium, *Statistically significant $P$<0.05

**Table 3:** Haplotype association statistics of *MTHFR* polymorphisms

| Gene | Haplotype | Freq. | $\chi^2$ | P-value |
|---|---|---|---|---|
| *MTHFR* | 11 | 0.429 | 0.377 | 0.539 |
| | 21 | 0.418 | 0.069 | 0.793 |
| | 22 | 0.125 | 0.833 | 0.361 |
| | 12 | 0.027 | 0.615 | 0.432 |

1 denotes the first nucleotide of the polymorphism and 2 denotes the second; eg. in *MTHFR* C677T, C is represented by 1 and T by 2. Similarly, for *MTHFR*, A1298C, A is represented by 1 and C by 2. The order of SNP is *MTHFR* A1298C and *MTHFR* C677T.

Sub-phenotype analysis (Table 4) showed that age-at-onset (AAO) of MI was affected by *MTHFR* C677T (TT=39y vs CT/CC=49y, $P$=0.006) polymorphism. This indicates that 677TT genotype appears to contribute to early development of atherosclerosis leading to MI at a younger age. This is further strengthened by the observation that homocysteine levels are associated with *MTHFR* C677T (TT=33 mg/dl, CT/CC=18 mg/dl) as previously reported in the literature[6].

An association was found with the *MTHFR* A1298C heterozygote and hypertriglyceridemia (TAG=184±127 mg/dl) which we consider to be due to stratification as the TAG levels of both homozygotes (AA and CC) were lower (162±86, 151±72 mg/dl, respectively). In any case, TAG levels were not seriously elevated in our sample population.

**Table 4:** Association statistics of *MTHFR* polymorphisms with sub-phenotypes.

| SNP | Age | BMI | Glucose | CHL | TAG | HDL | LDL | HCY |
|---|---|---|---|---|---|---|---|---|
| *MTHFR* | | | | | | | | |
| A1298C | 0.982 | 0.105 | 0.043 | 0.106 | 0.005* | 0.202 | 0.558 | 0.159 |
| C677T | 0.006* | 0.212 | 0.912 | 0.324 | 0.668 | 0.631 | 0.485 | <0.001* |

BMI: Body mass index (kg/m$^2$), CHL: Cholesterol (mg/dl), TAG: Triglycerides (mg/dl), HDL: High density lipoprotein (mg/dl), LDL: low density lipoprotein (mg/dl), HCY: Homocysteine (µmol/l), *Statistically significant $P$<0.05 by ANOVA

## DISCUSSION

There is an epidemic of heart disease in Pakistan[12]. The disease manifests itself at a relatively younger age in Pakistani population. A comprehensive survey carried out across the country revealed that Pakistani MI patients were at least 10 years younger than patients in the West[13].

In addition to traditional risk factors for CHD such as, diabetes mellitus, hypertension, dyslipidemia, obesity, hyperhomocysteinemia has also been considered as another risk factor for the development of atherosclerosis[1]. Since mild hyperhomocysteinemia is highly prevalent in Pakistan population[14], it is possible that mutations in enzymes involved in homocysteine metabolism, especially, *MTHFR* might be associated with high rates of CHD in this population. However, several studies focusing on *MTHFR* C677T polymorphism have reported conflicting results. A meta-analysis of 40 observational studies showed a significant association between *MTHFR* 677T genotype and increased risk for developing CHD, especially in individuals with low folate[15]. Similarly in a Tunisian Arab population, *MTHFR* 677TT has been reported to be associated with coronary artery disease[16]. Contrary to these observations, we have reported that while homozygosity for *MTHFR* 677T was associated with hyperhomocysteinemia, it did not appear to be a determinant of increased risk for AMI in Pakistani population[6]. Only a few studies have investigated the association between two *MTHFR* polymorphisms and their haplotypes with CHD. In the present study, *MTHFR* A1298C polymorphism along with *MTHFR* C677T polymorphism did not show any association with either plasma homocysteine or AMI in Pakistani population.

It was noteworthy that 1298CC genotype was present in appreciable proportion (26.9%) in this population. Similar findings have been reported by Kumar et al who have shown variant allele frequency to be 0.44 in Indian population[7]. Lack of association between *MTHFR* A1298C (individually or combined with *MTHFR* C677T) and coronary artery disease in Italian and Arab populations has also been reported earlier[17,18].

Age at onset (AAO) of MI was 10-year younger in homozygous variants (677TT) compared to 677CC/CT. This is consistent with previous studies which have shown onset of MI in younger groups with 677TT genotype[19]. It should also be noted that homozygous variant 677TT is not very prevalent in the Pakistani population (1-3%). This is in line with the findings of previous reports showing that compared to Caucasians, South Asian people have a relatively low prevalence of 677TT genotype[4,6,8]. It is therefore, conceivable that *MTHFR* polymorphism C677T does not have any relationship with MI in this population. Moreover, co-occurrence of *MTHFR* 677TT/*MTHFR 1298* CC does not appear to have any influence on either plasma homocysteine levels or AMI in this population. While paraoxonase gene (*PON* gene) cluster is associated with MI in Pakistani subjects[20], *MTHFR* gene appears not to have any role in it.

## CONCLUSION

It is suggested that increased incidence of AMI in Pakistan population does not involve pathways related to *MTHFR*. It is, therefore, imperative that for unraveling the genetic basis of CHD in Pakistani patients, polymorphisms in other homocysteine related enzymes should be investigated.

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