Common Polymorphism A1298C in Methylenetetrahydrofolate Reductase Gene Is not a Risk Factor for Coronary Artery Disease in Selected Iranian Patients

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Abstract

Background: Coronary artery disease (CAD) is emerging as a major public health concern in most developing countries. During the past 10 years, the vast majority of over 100 case-control retrospective studies have shown that elevated plasma homocysteine level is a strong independent risk factor for coronary artery disease. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. A second polymorphism, A1298C, in MTHFR gene, is reported to be associated with decreased enzyme activity and may give rise to elevated blood homocysteine level and increased risk of coronary artery disease.

Methods: In the present study we used PCR-RFLP analysis to investigate the association between A1298C polymorphism and blood homocysteine level and the risk of CAD in 100 patients compared to 100 normal controls.

Results: The frequency of mutated allele and genotype distribution showed no significant difference between patient and control groups. Although the elevated level in blood homocysteine were observed in Iranian CAD cases compared to the normal control, the A1298C polymorphism was not associated with increased CAD risk in studied population as supported by a P value>0.05 and chi-square equal to 0.697.

Conclusion: An increased plasma homocysteine concentration confers an independent risk factor for CAD. Although A1298C polymorphism in MTHFR gene has effects on enzyme activity but our findings do not support a major role for this polymorphism in homocysteine metabolism and it can not be considered a major risk factor for coronary artery disease in a selected Iranian population.

Keywords: MTHFR • Polymorphism • Homocysteine • Folate

Introduction

Coronary artery disease (CAD) is the major cause of death in industrial nations. Despite advances in our understanding of cardiovascular disease, traditional risk factors such as hypertension, smoking, diabetes mellitus, and dislipidemia do not accurately predict cardiovascular events.¹³ Homocysteine is an emerging new risk factor for CAD. Mild-to-moderate hyperhomocysteinemia is a well-established independent risk factor for coronary cerebral and peripheral atherosclerotic diseases and venous thrombosis.⁴⁶ Numerous clinical studies have shown that total homocysteine is a risk factor for CAD and stroke in humans and predicts mortality independently of traditional risk factors.
factors in patients with CAD.7,8

Until recently, the normal range for homocysteine was considered to be 5 to 15 μmol/L. It is now widely accepted that upper range of normal may be 10 to 12 μmol/L for middle-aged adults and that risk for cardiovascular disease occurs if plasma homocysteine exceed this value. Patients with CAD and other cardiovascular diseases usually have mild hyperhomocysteinemia (>12 to 25 μmol/L) with an incidence of 30 to 50 percent.9

Plasma homocysteine concentration is determined by both genetic and nutritional factors. Deficiencies of folate, vitamin B12, or B6 can lead to impaired homocysteine metabolism and hyperhomocysteinemia.5-11 In addition, mutations in the genes coding for methylenetetrahydrofolate reductase (MTHFR), methionine synthase, and cystathionine B synthase may also produce hyperhomocysteinemia. Smoking, excessive coffee consumption, and lack of exercise are associated with elevation in homocysteine as well.12,13 Because homocysteine has a thiol, it can undergo auto-oxidation and oxidation with other thiols. The resulting reactive oxygen species, hydrogen peroxide and superoxide anion radical, generate oxidative stress and induce vascular dysfunction.14,15 Recent evidence suggests that homocysteine may limit the bioavailability of nitric oxide, resulting in the impairment of folate-mediated vasodilation. The limited bioavailability of nitric oxide could be due to nitrosothiol formation with homocysteine. Homocysteine may also target specific proteins and impair their activity and function through disulfide bond formation.16

Homocysteine is a thiol compound derived from methionine that can be metabolized by two pathways; folate and cobalamin dependent remethylation to methionine or transsulfuration to cysteine with ultimate degradation.5-7 In both pathways, major genetic defects or enzyme deficiencies are associated with high plasma homocysteine.7,8,17 A less frequent form of mild hyperhomocysteinemia is genetic defect in methylenetetrahydrofolate reductase (MTHFR), a key enzyme regulating folate metabolism. MTHFR catalyses the reduction of 5,10 Methylenetetrahydrofolate to 5, Methylenetetrahydrofolate, which donates a methyl group to homocysteine to be transformed into methionine.18,19 The MTHFR gene is located on chromosome 1. There are two well-described and commonly occurring polymorphisms in the MTHFR gene: C677T and A1298C. The C677T polymorphism occurs in exon 4 and results in an alanine to valine substitution at codon 222. This polymorphism lies in the S-adenosylmethionine regulatory domain of the enzyme. The binding of the S-adenosylmethionine (SAM) results in conformational change within the MTHFR enzyme, which inhibits the enzyme’s activity.24,25

The A1298C polymorphism is associated with decreased enzyme activity and altered distribution of intracellular folate metabolites toward an accumulation of the 5,10 Methylenetetrahydrofolate, the precursor for thymidilate and purine synthesis.26-28 A decreased availability of 5-Methyltetrahydrofolate in remethylation homocysteine to methionine affects blood homocysteine and folate concentration.25

Only a few studies have investigated the relationship between the MTHFR A1298C polymorphism and the alteration of the risk of CAD. Some study reports that although this polymorphism affects the MTHFR activity, neither the homozygous nor the heterozygous status for A1298C polymorphism is associated with higher homocysteine or a lower plasma folate concentration.29,30 Other studies, however, have shown that A1298C mutation influences specific enzyme activity and homocysteine and folate concentration, but to a lesser extent than the C677T mutation.25,30 It is noteworthy that the subjects in the said studies had different ethnic backgrounds; consequently, whether the A1298C polymorphism has any relevance in other ethnic populations including Iran is still unclear. We speculate that a possible link exists between the MTHFR polymorphism and the occurrence of CAD, but with a smaller relative risk than the C677T polymorphism. Therefore, this study was designed to examine the prevalence of the MTHFR A1298C mutation and to assess the association between this polymorphism and plasma homocysteine level and CAD in a selected Iranian population.

**Methods**

Our case group was comprised of 100 patients (72 males and 28 females) at a mean age of 56.9 years (range 32-89 years) with angiographically documented CAD in Tehran Heart Center. A physician as well as cardiologists consulted all the chosen cases. The normal control group consisted of 100 healthy volunteers (61 males and 39 females) at a mean age of 45 years (range 32-57 years) without any clinical disorders selected from the general population in Tehran. Informed consent was obtained from all the patients and healthy subjects according to the guidelines of our ethics committee.

Venous blood samples were taken from fasting individuals (cases and controls) into EDTA vials. Total homocysteine was measured in plasma because the use of anticoagulant allows immediate sample processing. The samples were immediately ice packed and centrifuged within 30 min to avoid false increases of homocysteine due to release from red blood cells. Plasma samples were then refrigerated and
stored at -70 °C until analysis was done. The total plasma homocysteine concentration of the patients and normal controls was determined by using a homocysteine measuring kit via the Elisa method.

DNA was extracted from peripheral lymphocytes by standard salting out method as described by Miller et al.31 The MTHFR polymorphism genotype of A1298C was analyzed using polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP). The A1298C mutation alters a glutamate into alanine residue and abolishes the MboII restriction site. For the detection of the A1298C transition in the MTHFR gene ~100 ng DNA was used for PCR amplification. The PCR was carried out in a total volume 50μl, containing 50 ng of the forwardL primerL5’ CTTGGGGAGCTGAAGGACTACTAC3’ and 50 ng of the reverse primerL5’ CACTTTTGACCATTCCGGTTTG3’. 200 μM each dNTP, 10mM Tris-Hcl (PH=8.3), 50mM KCl, 3mM MgCl2, and 1 unit Taq polymerase. PCR parameters for the detection of the A1298C transition were as follows: an initial denaturation step of 5 min at 95°C, followed by 32 cycles of 95°C/60s (denaturation), 63°C/60s (annealing) and 72°C/45s (extension) and final extension for 10 min at 72°C to ensure a complete extension of all PCR products. The amplified PCR fragment of 163bp was digested with restriction enzyme MboII, followed by gel electrophoresis on 12% poly acrylamide. The mutated homozygous variant produced 4 fragments of 84, 31, 30, and 28bp, while 1298AC heterozygote produced 5 fragments of 84, 56, 31, 30, and 28bp and 1298AA wild-type produced 4 fragments of 56, 31, 30, and 28 bp (Figure 1).

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Descriptive values were expressed as mean±SD. Allele frequencies were calculated for each genotype by allele counting. Comparison of allele frequencies between the case and control groups was made using the Chi-square test via SPSS for Windows version 10 (Chicago, Illinois software). Differences between the patient and control groups were assessed with the student’s t-test for continuous variables (homocysteine). The Fisher exact test was used when the number of observations in any group was less than or equal to 5. All the tests were two-tailed, and p<0.05 was considered significant.

**Results**

One hundred CAD cases and 100 normal controls were genotyped for common mutation A1298C MTHFR. Their blood homocysteine level was measured to determine its linkage with CAD. The mean age of the cases and controls was 56.9 and 45 years respectively; 28% of cases and 39% of controls were woman. The frequencies of 1298AA, 1298AC, and 1298CC genotypes were 30%, 52%, and 18% in the CAD patients; and 35%, 50 %, and 15% in the controls. The frequencies of mutated allele (C) and normal allele (A) in patient and control groups were 44%, 56%, 40 %, and 60%, respectively. The difference between two groups was not significant (χ²=0.697, P>0.05). The prevalence of the A1298C polymorphism in the CAD patients and normal controls was compared with each other (Table 1).

| Table 1. Distribution of A1298C genotype and allele’s frequencies among cases and control group |
|---------------------------------|-----------|-----------|-----------|-----------|
|                                 | CC        | AC        | AA        | [%]A      | [%]C      |
| Cases                           | 18        | 52        | 30        | 56        | 44        |
| Controls                        | 15        | 50        | 35        | 60        | 40        |
| Heterozygous A/C, Homozygous mutant C/C, Normal A/A |

There was no significant difference in the incidence of A1298C MTHFR genotype between the males and females in either group (in patients χ²=1.64, P>0.05 and in controls χ²=5.61, P = 0.07). There was also no significant difference in the incidence of this polymorphism among different age groups (P>0.05). Plasma homocysteine concentration in both case and control groups was measured. The proportion of subjects with moderate hyperhomocysteinemia was substantially higher among the CAD patients compared to the controls. By comparison with the controls, the cases had higher plasma homocysteine concentration and the difference between the two groups was significant (15.56±6.77 versus 11.51±4.63, P<0.05).

The correlation between homocysteine levels with the MTHFR genotype was studied. The average of plasma homocysteine in CC, AC, and AA genotypes in the cases was 15.73±6.87, 15.08±5.51, and 14.68±6.81 and 12.81±5.30,
11.81±4.81 and 10.61±2.86 in the control group respectively (Table 2). There was no significant difference between the plasma homocysteine level in CC and AC genotypes in comparison with the AA genotype in both case and control groups.

Table 2. Distribution of plasma homocysteine concentration (μmol) among different A1298C MTHFR genotypes in case and control groups*

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Homocysteine concentration (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (100)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>15.73±6.87</td>
</tr>
<tr>
<td>AC</td>
<td>15.08±5.51</td>
</tr>
<tr>
<td>AA</td>
<td>14.68±6.81</td>
</tr>
<tr>
<td>Controls (100)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>12.81±50.30</td>
</tr>
<tr>
<td>AC</td>
<td>11.81±4.81</td>
</tr>
<tr>
<td>AA</td>
<td>10.61±2.68</td>
</tr>
</tbody>
</table>

*Data are presented as mean±SD

Discussion

Over the past decade, the role of genetic polymorphisms of enzymes in folate metabolism has attracted much interest in epidemiological research on cardiovascular disease. Numerous studies have demonstrated a significant relationship between elevated plasma homocysteine concentration and CAD.25,29 Of several enzyme defects and deficiencies that can lead to elevated plasma homocysteine, polymorphism in MTHFR gene is reflected in enzyme activity, it has no significant effect on plasma homocysteine level and cannot be considered a major factor for CAD. The present findings are limited in that they were obtained from a relatively small study population. It means that some of our insignificant findings may be due to weak statistical power, and a small difference, if any, may not be detected with this sample size. Therefore, further investigation into the association between this polymorphism and risk of CAD is warranted and should include a larger sample size and other polymorphisms in folate metabolism and address interactions with folate status.

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References

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