Analysis of Relation Between C677T Genotype in MTHFR Gene and Prostatic Cancer in Iranian Males

Pezhman Fard-Esfahani1, Peyman Mohammadi Torbati2, Zahra Hashemi3, Shima Fayaz1, and Majid Golkar4

1 Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
2 Department of Pathology, Labbafi-Nezhad Hospital, Shahid Beheshti Medical University, Tehran, Iran
3 Department of Pathology, Shahid Beheshti Medical University, Tehran, Iran
4 Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran

Received: 29 Oct. 2011; Received in revised form: 26 Dec. 2011; Accepted: 26 Oct. 2012

Abstract- Methylenetetrahydrofolate reductase (MTHFR) enzyme is one of the most important enzymes with a pivotal role in the folate metabolism and DNA synthesis pathways. Single nucleotide polymorphism (SNPs) in the coding gene has been related to many medical diseases as well as diverse malignancies including the prostate cancer which is the leading cause of the cancer deaths in men and one of the major public health problems. The goal of this study is to determine the relationship between the MTHFR C677T SNP and the prostate adenocarcinoma in Iranian males attending to the Labbafi-nezhad hospital in Tehran. In this Case-control unmatched study, 67 and 75 paraffinized tissue samples were taken out of the specimens diagnosed previously as the prostatic adenocarcinoma and nodular prostatic hyperplasia for the case and control groups respectively. MTHFR C677T genotyping was done by the use of multiplex ARMS-PCR and frequencies of the alleles were compared between the case and control groups as well as calculating the deviation from Hardy-Weinberg equilibrium and Odds Ratio for the “T” allele regarding the prostatic carcinoma. The observed rates in the control group were not too different from that of expected from Hardy-Weinberg equilibrium (P=0.407). Frequencies of the possible genotypes were as follows: CC, 43.28% vs. 42.67%; CT, 49.25% vs. 52% and CT, 7.46% vs. 5.33% in the case and control groups respectively (P=0.85). 1.37 times increased risk was found for the homozygote carriers of C677T variant (OR: 1.37, 95% CI: 0.33-5.6; P=0.653) which is however statistically not significant. No association has been evident between the MTHFR 677C>T polymorphism and the risk of prostatic carcinoma in this study confirming the findings of some of the previous attempts; however, (OR: 1.37, 95% CI: 0.33-5.6) implies a slight effect of the homozygote on the carcinogenesis. Thus larger studies especially with a greater number of the samples are recommended.

© 2012 Tehran University of Medical Sciences. All rights reserved.

Keywords: MTHFR; Polymorphism; Prostatic carcinoma

Introduction

Methylenetetrahydrofolate reductase (MTHFR) enzyme is one of the most important enzymes with a pivotal role in the folate metabolism and DNA synthesis pathways. The enzyme activity is achieved by catalysing the formation of 5-MTHF which is the main circulating form of the folate in the human body serving as the chief donor of the “methyl” group to the homocysteine, the critical amino acid in folate-related DNA handling processes. Distinct single nucleotide polymorphisms of the MTHFR gene have been well recognized out of which the C677T is the most common (1), located at 1p36.6 (2) and known by the reference code of “rs-181133” (3). Change of C to T nucleotide causes subsequent substitution of valine instead of alanine in the enzyme protein structure that leads to thermo-lability and thus less functioning state of the enzyme resulting in DNA hypomethylation and inappropriate uracil incorporation both in turn affecting the cellular growth and proliferation pathways as well as playing role in the carcinogenesis (4). The relationship of MTHFR C677T polymorphism with many medical diseases has been investigated for years as well as with
diverse neoplastic conditions among which the prostatic adenocarcinoma is one of the most common public health problems involving one out of every six men being the leading cause of cancer deaths worldwide (5). It has been studied for years in relation to the above mentioned polymorphism with conflicting and sometimes paradoxical results, thus sounding to deserve more related researches. In this study MTHFR C677T polymorphism was investigated in relation to the prostatic adenocarcinoma.

Materials and Methods

Unmatched Case-Control study was designated with regard to the previous literature. Considering the average frequency of the disease (involvement of one out of every six men by prostatic carcinoma) and the rate of MTHFR C677T polymorphism in the population which was reported between 20 to 32% (mean: 26%) (1,6), 69 specimens were needed according to Quanto software. Case and Control groups were then collected referring to the Labbafi-Nezhad hospital pathology department archive and selecting 67 and 75 specimens diagnosed since 2006 to 2010 as prostatic adenocarcinoma and nodular prostatic hyperplasia respectively. All prepared slides were reviewed by two pathologists to select appropriate ones followed by sectioning the corresponding paraffin-embedded blocks, into 3-5 micrometer slices. DNA-extraction of the tissue on both groups was done by Rima Kit (reference No.: M-1214) and genotyping of the MTHFR C677T polymorphism was carried out via previously defined multiplex ARMS-PCR method using PCR conditions described elsewhere (7) and primer sets modified by inosine nucleotides in order to minimize non-specific reactions as shown in table-1. Reaction patterns have been summarized schematically in figure 1. Finally statistical analysis of the data was done by Chi-square test comparing the frequency of each allele in the groups between which the odds ratio of the calculated T-allele frequency with regard to the prostatic cancer was also compared. In order to confirm appropriate non-carcinomatous population sampling, the deviation of observed genotype frequencies was calculated using student t-test from the expected values found by Hardy-Weinberg equilibrium.

Sample: MUTANT/WILD

![PCR reaction patterns](image_url)

**Figure 1.** Schematic presentation of PCR reaction patterns.
Table 1. Primers used for MTHFR 677C>T genotyping.

<table>
<thead>
<tr>
<th>Assignation</th>
<th>Nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>CTGAAGCACTTGAAGGAGGAAGGIIIIICGGGAGC</td>
</tr>
<tr>
<td>CR</td>
<td>GACAGCCACCTTGGGAAACIIIIITTAAAGCAGG</td>
</tr>
<tr>
<td>TF</td>
<td>CATCTCTGGGTCAGAAGCAIIIIIGTCATGAGCC</td>
</tr>
<tr>
<td>TR</td>
<td>AGCCTCAAAGAAAAGCTGCGIIIIIATGAAATCGA</td>
</tr>
</tbody>
</table>

Figure 2. Electrophoreses gel representative of some specimen genotypes. Lanes 2, 4, 6-11: Homozygote CC; lanes 1 and 5: Heterozygote CT; and lane 3: Homozygote TT.

Table 2. Age distribution of the case and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prostatic Carcinoma (n=67)</th>
<th>BPH (n=75)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  %</td>
<td>n  %</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>0.057</td>
</tr>
<tr>
<td>&lt;=60</td>
<td>28 41.79%</td>
<td>20 26.66%</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>39 58.21%</td>
<td>55 73.34%</td>
<td></td>
</tr>
</tbody>
</table>

* P-value<0.05 is significant

Observed genotype frequencies in the control groups were not significantly different from the expected rates based on Hardy-Weinberg equilibrium (χ²=1.86, P=0.407, significance level: less than 3.84).

Age distribution of the patients in the case and control groups have been shown in table 2, revealing no noticeable age biases (P=0.057).

Table 3. Frequency of genotypes in the case and control groups.

<table>
<thead>
<tr>
<th>Genotype of MTHFR C677T</th>
<th>Prostatic Carcinoma</th>
<th>BPH</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  %</td>
<td>n  %</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>29 43.28</td>
<td>32 42.67</td>
<td>0.85</td>
</tr>
<tr>
<td>CT</td>
<td>33 49.25</td>
<td>39 52</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>5 7.46</td>
<td>4 5.33</td>
<td></td>
</tr>
</tbody>
</table>

* P-value < 0.05 is significant

Genotype frequencies were calculated in the case and control groups as below (Table 3), followed by measuring the mutant T-allele frequency which is 0.32 in prostatic cancer patients and 0.31 in the control group (Table 4), neither revealing any statistically significant differences (P=0.85 and 0.89 respectively).
**Table 4.** T-allele frequency in the case and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Prostatic carcinoma</th>
<th>BPH</th>
<th><em>P</em>-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T allele frequency</td>
<td>0.32</td>
<td>0.31</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* *P*-value < 0.05 is significant

**Table 5.** Odds ratio of genotypes in the case and control groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Crude OR (95% CI)</th>
<th><em>P</em>-value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT vs. CC</td>
<td>1.37 (0.33-5.6)</td>
<td>0.6533</td>
</tr>
<tr>
<td>(TT or CT) vs. CC*</td>
<td>1.43 (0.37-5.6)</td>
<td>0.603</td>
</tr>
<tr>
<td>TT vs. (CT or CC)**</td>
<td>0.97 (0.50-1.9)</td>
<td>0.941</td>
</tr>
</tbody>
</table>

* *P*-value < 0.05 is significant ** Dominant T allele ** Recessive T allele

Also odds ratio (OR) for the T-allele was defined with regard to the occurrence of the prostatic carcinoma both in dominant and recessive forms which have been summarized in table 5; 1.37 fold increased risk of the homozygote TT genotype is seen which is however statistically insignificant (OR: 1.37, 95% CI: 0.33-5.6, *P*=0.653).

**Discussion**

The relationship of MTHFR C677T polymorphism with many medical diseases has been investigated for years as well as with diverse neoplastic conditions; Increased risk of the cardiovascular disease, essential hypertension and cerebrovascular accident (8-11) even in the children (12-16) are notable among the former as are documented hypercoagulability state and recurrent fetal losses (17-19), congenital anomalies (20) increased risk of Down syndrome (21-23) cognitive and behavioral diseases (24-27) and many metabolic alterations including diabetic nephropathy, Wilson’s encephalopathy, etc (28-30).

Many types of malignancies have been found associated with MTHFR C677>T polymorphism among which CML, AML, ALL and multiple myeloma (31,32), esophageal gastric and colorectal cancers (33-35), pancreatobiliary tumors (36,37), squamous cell carcinoma of head and neck and the lungs (38,39) and differentiated thyroid carcinomas (7) can be mentioned. However, squamous or basal cell carcinoma of the skin, urinary bladder cancers and ovaries tumor seems to be unrelated (40-42). In this study MTHFR C677T polymorphism was investigated in relation to the prostatic adenocarcinoma.

This study was designated as a case-control model which has been commonly used in the previous literature (43-50), choosing adequate sample number with the help of the Quanto software regarding the mean 26% frequency rate of the mentioned polymorphism in the population.

Paraffin-embedded tissue blocks were used for genotyping by the multiplex ARMS-PCR method. Review of the literature reveals similar approaches (43-50) with one study working on peripheral blood leukocytes instead of the prostatic tissue blocks (51) looking good as an alternative, as well as restriction fragment length polymorphism (RFLP) application in two researches (38,46) which gives rise to quite consistent results according to Ye and Dhillon (44).

Method of data analysis also seems to be comparable with all previous articles (43-50) using the odds ratio (OR) for the mutant allele in both homo- and heterozygote states with regard to the prostatic carcinoma. Also calculating the deviation of the genotypes from the Hardy-Weinberg equilibrium serves as a reliable and logistic method in studying the association of diseases and genetic polymorphisms (45-47).

Considering the results, review of the literature reveals comparable findings most of the time; Collin found no relationship between the MTHFR C677T polymorphism and the prostate cancer, neither in homo- nor in heterozygote states after reviewing eight known polymorphism including the MTHFR C677T in a large meta-analysis of twelve studies on more than 10,000 carcinoma and 40,000 control specimens (51). So have concluded Kimura Cicek and Relijic (47,48,39) as well as some else during 2000 to 2007 after which some conflicting data has been on public Marchal insists on the MTHFR C677T polymorphism as the only one associated with the prostatic carcinoma (OR: 2.9, 95% CI: 1.46-3.3); In 2010 this relationship has been shown by Safavinezhad in Iranian male population with referring to the possible role of folate supplementation preventive therapy (53) which has been confirmed also by Wu, et al in Taiwan (54) who have found MTHFR C677T SNP detection useful for anticancer interventions. In conclusion, in our study the relative age distribution of the case & control groups is rather comparable and no significant deviation from the Hardy-Weinberg equilibrium is noted. Frequency of the mutant T-allele is not statistically too different between the case and control groups; however, the Odds Ratio may be
indicative of partial increased risk of the homozygote individuals for developing adenocarcinoma of the prostate; thus further studies are recommended with a greater number of the specimens which might confirm the findings.

References


MTHFR gene polymorphism and prostatic cancer