Association between Mismatch Repair Gene MSH3 codons 1036 and 222 Polymorphisms and Sporadic Prostate Cancer in the Iranian Population

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Abstract

The mismatch repair system (MMR) is a post-replicative DNA repair mechanism whose defects can lead to cancer. The MSH3 protein is an essential component of the system. We postulated that MSH3 gene polymorphisms might therefore be associated with prostate cancer (PC). We studied MSH3 codon 222 and MSH3 codon 1036 polymorphisms in a group of Iranian sporadic PC patients. A total of 60 controls and 18 patients were assessed using the polymerase chain reaction and single strand conformational polymorphism. For comparing the genotype frequencies of patients and controls the chi-square test was applied. The obtained result indicated that there was significantly association between G/A genotype of MSH3 codon 222 and G/G genotype of MSH3 codon 1036 with an increased PC risk (P=0.012 and P=0.02 respectively). Our results demonstrated that MSH3 codon 222 and MSH3 codon 1036 polymorphisms may be risk factors for sporadic prostate cancer in the Iranian population.

Keywords: Sporadic prostate cancer - polymorphism - DNA mismatch repair - MSH3 - Iran
polymorphism may be a risk factor for prostate cancer (Hiroshi et al., 2008).

In this study we investigated the relationship between MSH3 codon 222 and MSH3 codon 1036 polymorphism and prostate cancer in a subset of Iranian population.

Materials and Methods

Samples

Genomic DNA of 18 patients with pathologically confirmed PC and 60 controls was extracted from peripheral blood samples using DNG™ plus DNA extraction kit (Cinnagen, Iran).

PCR amplification

Polymerase Chain Reaction - Single Strand Conformational Polymorphism (PCR-SSCP) technique was applied for studying the MSH3 codon 222 and MSH3 codon 1036 polymorphism. Primer sequences were according to the Hiroshi et al. (2008). PCR amplifications were carried out in 50 μl volumes using the following cycling program: initial denaturation at 95°C for 5 min followed by 30 and 25 cycles of denaturation for codon 1036 and codon 222 respectively at 95°C for 30 sec, annealing at specific annealing temperature (Table 1) for each polymorphism for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 7 min. Primer sequences and annealing temperatures are written in Table 1.

The PCR products were electrophoresed on agarose gel and analyzed by SSCP.

SSCP analysis and direct sequencing

Each non-denaturating polyacrylamide gel (10%) was prepared containing: 5 ml acrylamide-bisacrylamide 40% (ratio of acrylamide-bisacrylamide was 38:2), 2 ml TBE (10x), 13 ml distilled water, 200 μl APS (0.1%) and 20 μl TEMED. After polymerization of polyacrylamid gel for about 30 min, the gel was pre-run at 130 V for 10 min. Samples were prepared through mixing 10 μl PCR product with 3 μl loading dye, heated at 95°C for 5 min and then chilled on ice-bath and immediately loaded in wells. Electrophoresis performed at 70 V for 8 h at room temperature. DNA bands on gel were visualized by silver staining using standard methods (Sanguinetti et al., 1994). The PCR products were confirmed by direct sequencing.

Statistical analysis

Chi-Square was applied to assess significance of the observed differences in allele frequencies of the studied polymorphism of MSH3 codon 222 and MSH3 codon 1036 polymorphism between patients and controls. P<0.05 was considered significant.

Results

We analyzed PCR products of two codons of the MSH3 codon 222 and MSH3 codon 1036 by SSCP method to determine the relationship between MSH3 polymorphism and prostate cancer in 18 Iranian PC patients and 60 controls resident in Isfahan.

Data from this investigation indicated that there was statistical difference in the MSH3 codon 222 and MSH3 codon 1036 polymorphisms between cases and controls. The genotype distribution for MSH3 codon 222 in normal samples showed 71%, 22%, 7% for the G/G, G/A, A/A genotypes respectively and this percentage in patient group were 83%, 16%, 1%. The statistical analysis indicated that there was a significant difference between controls and patients in G/A genotype compared with two others ($\chi^2=6.250$, P=0.012). The distribution of A/A, A/G, G/G genotypes for MSH3 codon 1036 among cancer cases were 67%, 28%, 7% and in normal samples were 57%, 30%, 13% respectively. The obtained result for MSH3 codon 1036 also indicated that there was a significantly increase in G/G genotype between controls and patients samples ($\chi^2=5.44$, p=0.02). These results are summarized in Table 2.

Discussion

The mismatch repair system (MMR) is a post-replicative DNA repair mechanism which its defect can lead to rapid tumor progression via accumulated DNA damage and MSH3 protein has an important role in repair of mistakes. Until now, many studies were performed to determine the relationship between MSH3 gene polymorphism and different cancers.

The previous studies indicated the polymorphism of MSH3 gene is a risk factor in various cancers. For example Orimo et al found 3 SNPs in the MSH3 gene and the high frequency of the G693 (G/A) allele in patients with sporadic colon cancer (Orimo et al., 2000). Sonja et al. (2007) reported there was relationship between genetic variants in MSH3 and an increased risk of colorectal cancer. They also found that there were strongly associated between MSH3 940Q and 1036A variants and proximal colon cancer (Sonja et al., 2007). Investigation of the effect of MSH3 SNPs in ovarian cancer detected no associated

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Table 1. Primer Sequences and Annealing Temperature of MSH3 codon 222 and MSH3 codon 1036

<table>
<thead>
<tr>
<th>PCR Products bp</th>
<th>Primer sequence</th>
<th>Annealing Temperature (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>MSH3 codon222 rs1805335: S5-AAAACCTTTATACATTTTTTGTTGCG-3' AS5-ACTGCATTTTCTGTGGCTGTTG-3' MSH3 codon1036 rs26279: S5-TTCAGCTTCCAAGGACAGTTT-3' AS5-CTCTCACGCTTTTGGACTTG-3'</td>
<td>53</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>57</td>
</tr>
</tbody>
</table>

Table 2. MSH3 codon 222 and MSH3 codon 1036 Polymorphism in PC Patients and Controls

<table>
<thead>
<tr>
<th>Gene (genotype)</th>
<th>No.PC (%)</th>
<th>No.Co (%)</th>
<th>Mean±SE</th>
<th>SD</th>
<th>Chi-Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH3 codon 222:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>(83)</td>
<td>(71)</td>
<td>0/81±0/04</td>
<td>40</td>
<td>13.517</td>
<td>0.000</td>
</tr>
<tr>
<td>G/A</td>
<td>3 (16)</td>
<td>13 (22)</td>
<td>0/24±0/04</td>
<td>43</td>
<td>6.25</td>
<td>0.012</td>
</tr>
<tr>
<td>A/A</td>
<td>0 (1)</td>
<td>4 (7)</td>
<td>0/95±0/02</td>
<td>22</td>
<td>4.821</td>
<td>0.003</td>
</tr>
<tr>
<td>MSH3 codon 1036:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>12 (67)</td>
<td>34 (57)</td>
<td>0/70±0/05</td>
<td>46</td>
<td>10.522</td>
<td>0.001</td>
</tr>
<tr>
<td>A/G</td>
<td>5 (28)</td>
<td>18 (30)</td>
<td>0/41±0/06</td>
<td>49</td>
<td>7.348</td>
<td>0.007</td>
</tr>
<tr>
<td>G/G</td>
<td>1 (5)</td>
<td>8 (13)</td>
<td>0/88±0/04</td>
<td>32</td>
<td>5.444</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*No.PC=Number Prostate Cancer. No.Co=Number Control. SE=Standard Error
between MSH3 SNPs and risk of ovarian cancer (Song et al., 2006).

In an study, performed on prostate cancer by Hirata et al indicated that the MSH3 codon 222 and MSH3 codon 1036 polymorphism may be a risk factor for prostate cancer in Japanese men (Hiroshi et al., 2008). Our study also indicated a significant association between MSH3 polymorphism and prostate cancer. The frequency of G/A genotype of MSH3 codon 222 was significantly higher in patients than controls (P=0.012). Also a significantly increase in the G/G genotype of MSH3 codon 1036 was observed in cases compared to the controls (p=0.02).

Therefore MSH3 polymorphism can be considered as a risk factor for prostate cancer in Iranian population. This is the second report to show an association between MSH3 gene polymorphism and prostate cancer. Furthermore additional studies are needed to establish these results and to assess the role of MSH3 gene polymorphism in prostate cancer.

Acknowledgements

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References


