

RESEARCH ARTICLE

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

Fariba Jafary¹, Mansoor Salehi^{2*}, Maryam Sedghi³, Nayereh Nouri³, Farzaneh Jafary⁴, Farzaneh Sadeghi⁵, Shima Motamedi⁶, Maede Talebi¹

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

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Introduction

Prostate cancer is one of the most frequently diagnosed cancers among men and the second leading cause of cancer death especially in most Western populations (Crawford et al., 2003; Angie et al., 2005; Erika et al., 2011; Maurice et al., 2011). The main etiology of PC is still unknown so that it seems like that PC is a multifactorial disease associated with several risk factors such as ethnicity, family history, patient age, obesity and dietary constituents (Shukla et al., 2005; Liang et al., 2008; Ebru et al., 2009).

The mismatch repair system (MMR) is one of several DNA repair pathways that its defect can lead to cancer, a conserved process from *Escherichia coli* to mammals which is responsible to recognize and corrects insertion, deletion and mispaired bases during DNA replication errors and recombination (Brian et al., 2000; Iyer et al., 2006; Sung-Hoon et al., 2006; Kenji et al., 2010). The mismatch repair system consists of various types of proteins including MSH2, MSH3, MSH6, MLH1, PMS1, PMS2 and MLH3 that are responsible for repair of DNA replication errors. Deficiency of MMR proteins in cells lead to increase in the mutation rate or genomic instability that would end up uncontrolled cell proliferation (Paivi et al., 2001; Thomas et al., 2005; Huixian et al., 2007;

Xiaoqun et al., 2011).

In the mismatch repair system the MLH1 protein forms a heterodimer complex with PMS2 (MutLa). This heterodimeric complex binds to the heteroduplexes MutSa (composed of MSH2 and MSH6 which repair base-base mismatches or single extra bases) or MutSb (composed of MSH2 and MSH3 which recognizes large insertion-deletion loops) and is responsible for the recruitment section of DNA mistakes and replace it with correct DNA sequence (John et al., 1999; Wei et al., 2000; Mathonnet et al., 2003; Thomas et al., 2005; Iyer et al., 2006).

The MSH3 protein is one of the essential components of mismatch repair system that in humans encode by the MSH3 gene which is located on chromosome 5q11-13. It has 1137 amino acid residues that giving the molecular mass of approximately 128 kDa (Risinger et al., 1996). It has an important role in repair of mistakes which are created during DNA replication in cell division. Its heterodimer with MSH2 form MutS beta which binds to DNA mismatches and thereby initiating DNA repair (Winfried et al., 2000; Jens et al., 2004; Walter et al., 2011).

The result of investigation by Hiroshi et al. (2008) on association between polymorphism in mismatch repair genes and prostate cancer suggested that the MSH3

¹Young Researcher Club, I.A.U. Falavarjan University, ²Division of Genetics, Department of Biomedical Sciences, ³Department of Anatomical Sciences, Medical School, Isfahan University of Medical Sciences, Isfahan, ⁴Medical Genetics Laboratory, Alzahra Hospital, Medical University of Medical Sciences, Isfahan, ⁵Biology Department, Faculty of Basic Science, Alzahra University, Tehran, ⁶School of Veterinary Medicine, Razi University, Kermanshah, Iran *For correspondence: m_salehi@med.mui.ac.ir

polymorphism may be a risk factor for prostate cancer (Hiroshi et al., 2008).

In this study we investigated 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 1,030 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-denaturing 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 polyacrylamide

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

PCR Products (bp)	Annealing Temperature (C)	Primer sequence
178	53	MSH3 codon222 rs1805355: S 5-AAAACCTTATACATCTTTGGTTGC-3' AS 5-ACTGCATCTTTGTGCTGCTG-3'
200	57	MSH3 codon1036 rs26279: S 5-TTTCAGCTTTCAGGCACAGTT-3' AS 5-CCTCCAGCTCTTTGACTTG-3'

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

Gene (genotype)	No.PC (%)	No.Co (%)	Mean±SE	SD	Chi-Square	P Value
<i>MSH3</i> codon 222:						
G/G	15 (83)	43 (71)	0/81±0/04	0/40	13.517	0.000
G/A	3 (16)	13 (22)	0/24±0/04	0/43	6.25	0.012
A/A	0 (1)	4 (7)	0/95±0/02	0/22	4.821	0.003
<i>MSH3</i> codon 1036:						
A/A	12 (67)	34 (57)	0/70±0/05	0/46	10.522	0.001
A/G	5 (28)	18 (30)	0/41±0/06	0/49	7.348	0.007
G/G	1 (5)	8 (13)	0/88±0/04	0/32	5.444	0.020

*No.PC=Number Prostate Cancer. No.Co=Number Control. SE=Standard Error

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

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.

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