Genetic Polymorphism of Mismatch Repair Genes and Susceptibility to Prostate Cancer

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Purpose: Mismatch repair (MMR) is one of the DNA repair systems that correct mispaired bases during DNA replication errors. Polymorphisms in genes can increase susceptibility to the development of prostate cancer (PCa). In this study, we investigated mutL homolog 1 (MLH93- (1G>A (rs1800734) and mutS homolog 3 (MSH3) (rs26279) polymorphisms with the risk of PCa.

Materials and Methods: In this study of Iranian population, 175 histopathologically confirmed (PCa) patients and 230 benign prostate hyperplasia (BPH) as the controls were recruited. The genotypes of MLH1 and MSH3 were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: There was no significant difference of MLH1 ($P = 0.4$) and MSH3 ($P = 0.5$) genotype distributions among PCa cases and controls. And also patients with PCa were not significant differences compared to those without in stage of cancer, grade of tumor, perineural invasion, and vascular invasion.

Conclusion: Our results did not show adequate evidence for any significant association of MLH1 and MSH3 polymorphisms and PCa .

Keywords: prostate cancer; MSH3; MLH1; polymorphism; PCR-RFLP.

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed malignancy among aging males after skin cancer and is the sixth leading cancer resulting in mortality in males [(1)]. Although the reason of PCa is still unclear, epidemiological studies have suggested that it is a multifactorial disease with a genetic basis[(2)]. There are a number of studies being carried out to identify biomarkers in patients with high risk of adverse PCa outcomes[(3)]. DNA repair systems reduce any risks conferred by mutations from risk factors, including etiologic and environmental leading to exit somatic mutations that may be important for initiation of late onset diseases[(4)]. A highly conserved mismatch repair (MMR) functions to boost replication accuracy by correcting and deleting base pair mismatch during DNA replication[(5)]. The MMR system consists of seven mismatch repair genes, including MSH2, MSH3, MSH6, MLH1, PMS1, PMS2, and MLH3. The heterodimers which are formed by MSH2-MSH6 (MutSα) and MSH2-MSH3 (MutSß) mismatch repair complexes identify mispaired bases. The function of MutSα complex is to investigate and repair the base-base and insertion/deletion (I/D) mispairs. MutSα is also likely to be associated with another heterodimer of MLH1 and PMS2 (MutLα) [(6)]. Defects in this MMR pathway result in a considerable rate of mutation or genetic instability, which in turn leads to variation in genes that regulate cell proliferation and death[(7)]. Numerous mutations and polymorphisms have been distinguished in MMR genes[(8)]. Genetic polymorphisms of mutL homolog 1 (MLH1) have a detrimental effect on the MMR capacity and cancer risk. The MLH1 gene, which is located on chromosome 3p contains 19 exons and it is shown to cover a region of up to 100 kilo bases (kb)[(9)]. The MLH1 -93G>A (rs1800734) polymorphism is closely linked to several cancers, including tobacco-related oral carcinoma, colorectal, and lung cancer [(10-12)]. The mutS homolog 3 (MSH3) protein acts as one of the important compo-

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Table 1. Demographic and behavioral characteristics of cases and controls

Factor		Prostate cancer	Healthy individuals	*P*-value
Smoking status				
No	No (%)	71 (57.72)	108 (81.82)	< 0.0001
Yes	No (%)	52 (42.28)	24 (18.18)	
Having 3 or more sons older than 40				
≤ 2	No (%)	72 (98.63)	103 (94.50)	0.1
>2	No (%)	1 (1.37)	6 (5.5)	
History of marriage				
No	No (%)	2 (1.94)	0	0.2
Yes	No (%)	101 (98.06)	130 (100)	
Family history				
No	No (%)	93 (87.74)	125 (98.43)	0.001
Yes	No (%)	13 (12.26)	2 (1.57)	
Age	mean (SD)	62.38 (7.59)	70 (8.64)	0.0001
BMI	mean (SD)	24.87(2.88)	24.74(3)	0.6

nents of the mismatch repair system, which is encoded by the MSH3 gene and located on chromosome 5q in humans. It possesses 1137 amino acid residues with the molecular mass of approximately 128 kilodaltons (kDa) [13]. Currently, scientists have reported at least 180 single nucleotide polymorphisms (SNPs) in MSH3 gene. Among all of these SNPs, rs26279 G>A polymorphism is frequently investigated and has been recently thought to be carcinogenic. Some studies showed that this polymorphism is associated with the risk of different types of cancer, including breast cancer, colorectal cancer, bladder cancer, PCa and ovarian cancer [14-18].
Considering the importance of MLH1 and MSH3 in the carcinogenic process, several case-control studies have been patients in patients in order to investigate the possible correlation between mentioned two polymorphisms and the risk of PCa.

METHODS

Sample collection

This case-control study consisted of 175 patients with PCa and 230 controls with benign prostatic hyperplasia (BPH). The patients were selected between February 2010 and April 2015 from the department of urology of Shahid Labbafinejad Medical center, Tehran, Iran. Written informed consent was obtained from all of the participants and details of the consent form was approved by the ethics committee.
Demographic and clinical data were collected, including age, body mass index (BMI), history of PCa in 1st-degree relatives, blood group, total and free prostate-specific antigen (PSA) level, staging and grading by questioners.
Open laparoscopic or radical prostatectomy was used to determine the tumor stage, grade, vascular and perineural invasion.
Tumor stage and tumor grade were determined by TNM staging (pathologic tumor stage, nodal invasion, metastasis) and the Gleason scoring (GS >7, GS ≤ 7) system, respectively [19,20].
The control group with BPH had to fulfill the following inclusion criteria for decreasing the likelihood of misdiagnosed prostate cancer:
1) Either serum PSA < 4.0 ng/mL or pathological reports of no malignancy of transrectral ultrasound-guided prostate biopsy if there was a serum PSA > 4.0 ng/mL.
2) Normal digital rectal examination.
3) Negative pathological report of malignancy in resected prostatic tissues from open surgical prostatectomy.
The exclusion criteria for patients with PCa were a family history of PCa in the control group, consuming any PSA decreasing medication, hormone therapy, orchiectomy and non-adenocarcinoma of the prostate.

DNA extraction and Genetic analysis

Peripheral blood samples from BPH and PCa were collected before surgery in a tube containing EDTA, and DNA extraction was performed by using DNGTM plus DNA extraction kit (Cinnagen, Iran) and maintained at +4°C. The rs1800734 polymorphism of MLH1 and rs26279 polymorphism of MSH3 genes was determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. PCR was performed in a total 50µl reaction volume

Table 2. Crude and adjusted associations between different polymorphisms and prostate cancer.

Gene (Polymorphism)	Genotypes	Cases No (%)	Controls No (%)	*P*-value	Crude OR(95% CI)	AdjustedOR(95% CI)
	AA	29 (16.76)	29 (12.61)	1		
MLH1 (rs 1800734)	AG	83 (47.98)	122 (53.04)	0.4	0.68 (0.38-1.22)	0.71 (0.30-1.72)
	GG	61 (35.26)	79 (34.35)		0.77 (0.42-1.43)	0.69 (0.28-1.68)
MSH3 (rs26279)	AA	82 (47.40)	99 (43.04)		1	
	AG	81 (46.82)	112 (48.70)	0.5	0.87 (0.58-1.31)	0.71 (0.36-1.38)
	GG	10 (5.78)	19 (8.26)		0.63 (0.28-1.44)	0.24 (0.06-0.97)

MLH1, mutL homolog 1; MSH3, mutS homolog 3

containing 2mM MgCl2, 25 mM KCl, 5 mM Tris-HCl (pH 8.4), 0.23 mM each of deoxyribonucleotide triphosphate (dNTP), and 1 unit of Taq polymerase. The primers used for amplification of MLH1 were forward 5' - TTT CAG CTT TCA GGC ACA GTT – 3' and reverse primer 5' - CCT TCC AGC TCT TTT GAC TT – 3'. The polymorphic site of the MSH3 gene was amplified by the use of each primer: forward primer, 5' - TTT CAG CTT TCA GGC ACA GTT – 3', and reverse primer, 5′ - CCT TCC AGC TCT TTT GAC TT – 3'. The cycling condition for MLH1 and MSH3 were 95°C for 5min of one cycle; 95°C for 1 min ,58°C (MLH1) and 55°C (MSH3) for 1min and 72°C for 1min for 35 cycles and final elongation cycle of 72°C for 5min.

After PCR, RFLP method was used for MLH1 and MSH3 with PvuII and HhaI restriction enzymes, respectively (Thermo V scientific), and PCR products were digested at 37°C for 16 hr.

The three genotypes were identified according to their size for both genes: MLH1 AA (373bp), GG (285+88bp), and AG (373+285+88bp), MSH3 AA (200bp), GG (151+49bp) and AG (200+151+49bp).

Statistical analysis

Categorical variables were compared between cases and controls using Chi-square test. The comparison of continues variables was conducted by Mann–Whitney *U* test and independent t-test. Crude and adjusted (adjusted for age, family history of PCa and smoking status) logistic regression models were used to investigate the association between genotypes and PCa. All data analyses were performed using STATA ver.11 software. P-value less than 0.05 were considered statistically significant.

RESULTS

Totally 405 subjects were recruited in the study, including 230 (56.79%) healthy subjects and 175 (43.21) patients suffering from PCa. Mean (SD) age of them was 66.57 (9.01) years.

Frequencies of smoking habit (42.28% vs. 18.18% respectively; $P < 0.0001$) and familial history of cancer (12.26% vs. 1.57% respectively; $P = 0.001$) were higher among prostatic cancer patients compared to healthy subjects. In addition, these patients were significantly younger than controls (mean age: 62.38 vs. 70 respectively; $P = 0.0001$). No significant differences were found between two groups regarding marital status ($P = 0.2$), having more than 40-year-old son ($P = 0.1$) and mean BMI ($P = 0.6$) (**Table 1**).

As illustrated in **Table 2**, frequencies of AG and GG genotypes of MLH1 polymorphism among patients with and without PCa were 47.98% vs. 53.04% and 35.26% vs. 34.35%, respectively ($P = 0.4$). Corresponding figures for AG and GG genotypes of MSH3 polymorphism were 46.82% vs. 48.70% and 5.78% vs. 8.26% respectively ($P = 0.5$).

Crude and adjusted odds ratios between the presence of AG genotype of MLH1 polymorphism and developing PCa were 0.68 ($P = 0.2$) and 0.72 ($P = 0.4$), respectively. Corresponding odds ratios for GG genotype were 0.77 ($P = 0.4$) and 0.69 ($P = 0.4$), respectively. Crude and adjusted odds ratios representing the effect of different genotypes of MSH3 polymorphism on developing cancer were 0.87 ($P = 0.5$) and 0.71 ($P = 0.3$) respectively, for AG genotype and 0.63 ($P = 0.3$) and 0.24 ($P = 0.04$), respectively for GG genotype (**Table 2**).

G allele of MLH1 polymorphisms was observed among 59.25% and 60.88% of cases and controls, respectively ($P = 0.6$). The odds ratio between the presence of this allele and PCa was 0.93 ($P = 0.6$). Moreover, 29.19% of cases and 32.61% of controls carried G allele of MSH3 polymorphism ($p = 0.3$). The odds ratio between the presence of this allele and PCa was 0.85 ($P = 0.3$).

Polymorphisms and staging of cancer

The frequencies of different genotypes of MLH1 polymorphism among patients with initial stages of cancer compare to those with advanced stages were 40.54% vs. 68% respectively for AG genotype and 37.84% vs. 24% respectively for GG genotype ($P = 0.1$). Corresponding figures for genotypes of MSH3 polymorphism were 48.65% vs. 52% respectively for AG genotype and 5.41% vs 4% respectively for GG genotype ($P = 1$) (**Table 3**).

Polymorphisms and grading of tumor

Table 3 represents that 45.87% of low tumor grade patients and 45.87% of high tumor grade patients had AG genotype of MLH1 polymorphism. Corresponding rates for GG genotype were 28.13% and 39.45% respectively ($P = 0.3$). Moreover, AG and GG genotypes of MSH3 polymorphism among patients with lower grade of the tumor were 45.31% and 9.38% respectively, while these genotypes were carried by 47.71% and 3.67% of patients with high-grade tumor respectively ($P = 0.3$) (**Table 3**).

Polymorphisms and perineural invasion of cancer

Among patients who developed perineural invasion, frequencies of AG and GG genotypes of MLH1 polymorphism were 50% and 31.43%, respectively, while corresponding frequencies for those without invasion were 41.67% and 31.43% Respectively ($P = 0.8$). In addition, 42.86% and 41.67% of patients with and without perineural invasion had AG genotype respectively, while, this genotype was presented among 8.57% and 8.33% of patients with and without perineural invasion respectively ($P = 1$) (**Table 3**).

Polymorphisms and vascular invasion of cancer

Only AG genotype of MLH1 polymorphism was found among patients with vascular invasion of the tumor (88.89%). Presence of AG and GG genotypes of this polymorphism was observed among 47.06% and 35.29% of patients without vascular invasion respectively ($P = 0.7$). The frequencies of the AG genotype of MSH3 polymorphism among patients with and without vascular invasion were 55.56% and 41.18% respectively. GG genotype was observed only among patients without vascular invasion of the tumor ($P = 0.4$) (Table 3).

DISCUSSION

PCa is one of the most common malignancy in males and leading causes of cancer mortality. There are few studies that are investigated the relationship between the MLH1 rs1800734 and MSH3 rs26279 polymorphisms and risk of PCa. MMR deficiency has been reported to be associated with increased risk of several types of cancer [21]. Until now, there are a large number of publications have demonstrated investigated the association of MLH1 and MSH3 polymorphisms

and cancer susceptibility. MSH3 rs26279 and MLH1 rs1800734 polymorphisms are most widely studied for their association with cancer risk among those genetic variations. However, the results of these studies were controversy. Berndt et al. and Muniz-Mendoza et al. founded that MSH3 rs26279 and MLH1 rs1800734 polymorphisms have been extensively investigated for their correlation with the risk of colorectal cancer[14,22]. In contrast, Smith et al. did not observe any significant association between MSH3 rs26279 polymorphism and the risk of breast cancer[17]. Chen H et al. reported that there was not persuasive evidence showing that SNPs of rs1800734 were related to colorectal cancer susceptibility[23].

In this study, we realized that GG genotype of MSH3 polymorphism was significantly less common among patients with PCa compared to patients with BPH. Additionally, we observed that there was not a remarkable relationship between presence of MLH1 and MSH3 polymorphisms and different characteristics of the tumor. Although homozygote and heterozygote genotypes of MLH1 polymorphism decreased the odds of developing PCa approximately 30%, these effects were not statistically significant. Therefore, this polymorphism is not a protective factor for PCa. Among different genotypes of MLH3 polymorphism, only homozygote genotype showed a significant association with PCa, so that the presence of this genotype caused a 76% decrease in the odds of cancer. The considerable change between crude and adjusted estimates indicates that factors such as age, family history of PCa and smoking status can confound this association. To assess the effect of MLH1 and MSH3 polymorphisms on different characteristics of PCa, we compared the frequencies of various genotypes of these polymorphisms between patients with different stages, grades and invasions of cancer. It was observed that patients with advanced phases of cancer and invasive situation of the tumor had higher rates of MLH1 polymorphism. However, these differences were not statistically significant. Genotypes of MSH3 polymorphism were relatively different among patients with various stages and grades of the tumor, although still, these differences were not significant. Therefore, these two polymorphisms cannot be considered as protective or risk factors for progression of the PCa.

A meta-analysis, which was done by Xu JL et al. suggested that MLH1 -93G >A polymorphism could be a possible biomarker of cancer susceptibility[24]. In a study which was carried out by Hiroshi Hirata et al. for the first the association of MSH3 gene polymorphisms in PCa was reported. These results suggested that the MSH3 polymorphism may be a risk factor for PCa [15]. Ting Wang et al. indicated that the MLH1 -93G>A polymorphism could contribute to individual susceptibility to colorectal cancer and act as a risk factor for microsatellite instability-colorectal cancer[25].

A pooling analysis of hMLH1 polymorphisms revealed that hMLH1 polymorphisms may be associated with cancer risk, especially in Asians[26].

A meta-analysis in 2015 showed that MSH3 rs26279 variant is associated with an increased risk of overall cancer[27].

According to our result, the MLH1 and MSH3 polymorphisms did not show any significant association with prostate cancer. We also found an association of smoking habits and familial history of cancer among prostatic cancer patients. In addition, these patients were significantly younger compared to healthy subjects. Moreover, investigations should be carried out to detect the exact effects of such genetic factors on developing PCa and its severity.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90.
2. Bostwick DG, Burke HB, Djakiew D, et al. Human prostate cancer risk factors. Cancer. 2004;101:2371-490.
3. Salinas CA, Koopmeiners JS, Kwon EM, et al. Clinical utility of five genetic variants for predicting prostate cancer risk and mortality. The Prostate. 2009;69:363-72.
4. Park JY, Huang Y, Sellers TA. Single nucleotide polymorphisms in DNA repair genes and prostate cancer risk. Cancer Epidemiol. 2009361-85.
5. Li G-M. Mechanisms and functions of DNA mismatch repair. Cell research. 2008;18:85-98.
6. Iyer RR, Pluciennik A, Burdett V, Modrich PL. DNA mismatch repair: functions and mechanisms. Chem Rev. 2006;106:302-23.
7. Kinzler KW, Vogelstein B. Gatekeepers and caretakers. Nature. 1997;386.
8. Peltomäki P, Vasen H. Mutations associated with HNPCC predisposition—update of ICG-HNPCC/INSiGHT mutation database. Dis Markers. 2004;20:269-76.
9. Han H-J, Maruyama M, Baba S, Park J-G, Nakamura Y. Genomic structure of human mismatch repair gene, hMLH1, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). Hum Mol Genet. 1995;4:237-42.
10. Jha R, Gaur P, Sharma SC, Das SN. Single nucleotide polymorphism in hMLH1 promoter and risk of tobacco-related oral carcinoma in high-risk Asian Indians. Gene. 2013;526:223-7.
11. Raptis S, Mrkonjic M, Green RC, et al. MLH1–93G> A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. J Natl Cancer Inst. 2007;99:463-74.
12. Shih C-M, Chen C-Y, Lee L, Kao W-T, Wang Y-C. A polymorphism in the hMLH1 gene (-93G⟶ A) associated with lung cancer susceptibility and prognosis. Int J Mol Med. 2010;25:165.
13. Risinger JI, Umar A, Boyd J, Berchuck A, Kunkel TA, Barrett JC. Mutation of MSH3 in endometrial cancer and evidence for its functional role in heteroduplex repair. Nat Genet. 1996;14:102-5.
14. Berndt SI, Platz EA, Fallin MD, Thuita LW,

Hoffman SC, Helzlsouer KJ. Mismatch repair polymorphisms and the risk of colorectal cancer. Int J Cancer. 2007;120:1548-54.

15. Hirata H, Hinoda Y, Kawamoto K, et al. Mismatch repair gene MSH3 polymorphism is associated with the risk of sporadic prostate cancer. J Urol. 2008;179:2020-4.
16. Kawakami T, Shiina H, Igawa M, et al. Inactivation of the hMSH3 mismatch repair gene in bladder cancer. Biochem Biophys Res Commun. 2004;325:934-42.
17. Smith TR, Levine EA, Freimanis RI, et al. Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. Carcinogenesis. 2008;29:2132-8.
18. Song H, Ramus SJ, Quaye L, et al. Common variants in mismatch repair genes and risk of invasive ovarian cancer. Carcinogenesis. 2006;27:2235-42.
19. Epstein JI, Allsbrook Jr WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. Am J Surg Pathol. 2005;29:1228-42.
20. Hoedemaeker RF, Vis AN, Van Der Kwast TH. Staging prostate cancer. Microscopy research and technique. 2000;51:423-9.
21. Xiao X, Melton DW, Gourley C. Mismatch repair deficiency in ovarian cancer—molecular characteristics and clinical implications. Gynecol Oncol. 2014;132:506-12.
22. Muniz-Mendoza R, Ayala-Madrigal M, Partida-Perez M, et al. MLH1 and XRCC1 polymorphisms in Mexican patients with colorectal cancer. Genet Mol Res. 2012;11:2315-20.
23. Chen YC, Hunter DJ. Molecular epidemiology of cancer. CA Cancer J Clin. 2005;55:45-54.
24. Xu J-L, Yin Z-Q, Huang M-D, et al. MLH1 polymorphisms and cancer risk: a meta-analysis based on 33 case-control studies. Asian Pac J Cancer Prev. 2012;13:901-7.
25. Wang T, Liu Y, Sima L, et al. Association between MLH1-93G> a polymorphism and risk of colorectal cancer. PloS One. 2012;7:e50449.
26. Li S, Zheng Y, Tian T, et al. Pooling-analysis on hMLH1 polymorphisms and cancer risk: evidence based on 31,484 cancer cases and 45,494 cancer-free controls. Oncotarget. 2017;8:93063.
27. Miao HK, Chen LP, Cai DP, Kong WJ, Xiao L, Lin J. MSH3 rs26279 polymorphism increases cancer risk: a meta-analysis. Int J Clin Exp Pathol. 2015;8:11060-7.