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R462Q Mutation in Prostate Cancer Specimens

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Background: A candidate gene for hereditary prostate cancer (PC), recently identified is the RNASEL gene on the chromosome loci 1q25. This gene mediates the apoptotic and antiviral activities of interferon. In some studies, a significant relationship has been reported between the chromosome 1q24-25 (HPC1) and prostate cancer risk, while some other studies did not approve.
Objectives: The aim of this study was to determine the association between R462Q mutation and prostate cancer in a cross-sectional study.
Patients and Methods: One hundred twenty one samples from 51 patients with sporadic PC and 70 patients with non-cancerous prostate were screened for the R462Q mutation. All samples were formalin-fixed and paraffin embedded. The samples were investigated by the use of amplification refractory mutation system (ARMS) PCR, followed by gel electrophoresis. To analyze the data the Fisher's exact and Chi-square tests were used. Statistical analyses were performed, using SPSS 17 software program.
Results: The present study findings showed that RR, RQ and QQ genotypes compromised 82, 14, and 4% of the cancerous samples and 87, 13 and 0 % of the non-cancerous samples, respectively.
Conclusions: We did not find any association between the RNASEL Arg462Gln polymorphism and prostate cancer. Based on these results the RNASEL Gln/Gln genotype does not play an important role in the etiology of sporadic prostate cancer, in the general population. However, additional studies with bigger sample sizes are needed to more clearly explain the role of RNASEL mutations in hereditary prostate cancer.

*Keywords:*Polymorphism, Genetic; Mutation; Prostatic Neoplasms

1. Background

The first defensive line against pathogens (viruses, bacteria, fungi, and parasites) is the innate immune system. Pathogens must cross physical barriers, including skin and mucosal tissues in the first step. If the pathogens cross these barriers, they will be recognized by the innate immune system. Then active anti-microbial responses are generated and pro-inflammatory cytokines and interferons (IFNs) are produced at the cellular level. A subset of genes, called IFNs stimulated genes (ISGs), mediate IFN effects of. The ISGs are implicated in anti-angiogenic, apoptotic, cell cycle inhibitory and anti-viral effects (1, 2). After the viral attack, innate immune responses by producing interferon- induced 2′-5′ oligo adenylate synthetase, which activates an endoribonuclease, called RNASEL. The RNASEL is expressed by the RNASEL gene localized on the chromosome 1q24-25 (HPC-1), a region for hereditary prostate cancer (3). In some studies a significant relationship has been demonstrated between the chromosome 1q24-25 (*HPC1*) and prostate cancer risk (4). RNASEL, a role playing gene in regulating cell proliferation, apoptosis and tumor-suppressor cell, is one the candidate genes within the HPC1 region (5). According to the close linkage of HPC1 to the RNASEL, it seems that RNase L could suppress one or more steps in prostate tumorigenesis and/or metastasis, directly or indirectly. One of the most frequently occurring cancers among men is prostate cancer. The highest rates are reported in the United States, Sweden, Australia, Canada and France (4). The rate of prostate cancer incidence in Iran is 9.6 (3.2 to 16.0) per 100000 and is significantly less than those in developed countries. The incidence rate is similar to the Eastern Mediterranean regions (6). Prostate cancer may appear in familial or sporadic versions. There are usually mutations in genes, like ElaC2, MSR1 and RNase L, in familial cancer, however, hereditary prostate cancer has a low prevalence (7). In relation to sporadic and familial prostate cancer, several variants in RNASEL (E262X, 471delAAAG, Arg462Gln, Asp541Glu) have been detected. A common missense variant, causing threefold reduction in enzymatic activity, is substitution of glutamine (Gln) by Arginine (Arg) at codon 462 (a G to A nucleotide transition at base pair 1385) (3).

2. Objectives

As found in the epidemiological studies, there were diverse associations between Arg462Gln polymorphism and prostate cancer in sporadic and familial prostate cases (3, 5, 8-10). Therefore, in this study we evaluated the

association between this polymorphism and prostate cancer in a cross-sectional study.

3. Patients and Methods

3.1. Tissue Specimens

We used a cross-sectional design, using samples of cancerous and non-cancerous prostate tissue at the baseline. All human samples used in this study were retrieved from the archive of the Pathology Service of the Shafa Hospital, Jundishapur University of Medical Sciences, Ahvaz, IR Iran and were related to Khuzestan province. These formalin-fixed and paraffin embedded samples had been collected from 2010 to 2011. The ethical approval code was 55.14.3.2011. Age, clinical parameters and geographical locations of samples were recorded but unfortunately it was not feasible to separate the familial cases from the sporadic cases, based on the available data. Our study population consisted of 121 males, divided into two groups of individuals, previously diagnosed with prostate cancer by histopathological examination (n = 51) and control individuals with non-cancerous prostate (prostatitis, hyperplasia) (n = 70). Pathologically confirmation of prostate cancer was considered as inclusion criteria, whereas samples from patients with cancers related to the other parts of the body were excluded from study.

3.2. Molecular Analysis

Each paraffin block was cut at 10μm and collected in 1.5 mL autoclaved plastic micro tubes) (9). Ten sections were placed in each micro tube. Deparaffinization was performed with heating procedure as follow: a total of 300 μL universal buffer solution (H_3BO_3, diethyl barbituric acid, citric acid and KH_2PO_4 28.6 mM for all) at pH 9 was added to each micro tube, containing tissue sections and heated at 120 °C, using autoclave (Iran Tolid-110N) for 20 minutes. Then the samples were cooled for five minutes. The solid paraffin wax ring, formed above the buffer, was removed (10). DNA was extracted by DNA extraction kit (Cinnagen, Iran). The RNASEL G1385A mutation analysis was carried out, using the amplification refractory mutation system (ARMS). ARMS, also known as allele specific PCR (ASPCR) or PCR amplification of specific alleles (PASA), is used for detection of known single-base substitutions or micro deletions/insertions (11). Primers described by Casey et al. were used in this study (Table 1). PCR was performed in a final volume of 15 μL for control gene containing: Taq polymerase (Cinnagen, Iran) (1 U), PCR buffer 10 x (1.5 μL), 10 mM dNTPs (0.3 μL), 50 mM MgCl2 (0.5 μL), 10 ρmol/μL forward primer (0.3 μL), 10 ρmol/μL reverse primer (0.3 μL) and the sample DNA (1 μL) and in a final volume of 15 μL for RNASEL gene containing: Taq polymerase (1 U), PCR buffer 10 × (1.5 μL), 10 mM dNTPs (0.3 μL), 50 mM MgCl2 (0.5 μL), 10 ρmol/uL forward primer (0.5 μL), 10 ρmol/uL reverse primer (0.5 μL) and round 1 PCR product (2 μL). Modified programs for control gene and RNASEL gene is described as follows, respectively:

Cycles: Seven minutes at 95°C; (30 sec 95°C, 30 sec 65°C and 40 sec 72°C) × 30 cycles; 10 minutes at 72°C.

Cycles: Five minutes at 95°C; (30 sec 94°C, 30 sec 58°C, 40 sec 72°C) × 30 cycles; 10 minutes at 72°C.

Electrophoresis was performed using 8% polyacrylamide gel (3.2 mL acrylamide 30%, 1.2 mL TBE 10X, 7.6 mL water, 200 μL APS 10% and 10 μL tetramethylethylenediamine [TEMED]) and 2% agarose. Two PCR products were sent to Bioneer Company for sequencing, for result accuracy investigation.

3.3. Statistical Analysis

Statistical analyses were performed, using SPSS 17 software program. Correlation between RNASEL gene type and prostate cancer was analyzed, using Fisher's exact test and Chi-square.

4. Results

We investigated whether the prostate cancer incidence is higher among carriers of the R462Q polymorphism of RNASEL. In total, 51 prostate cancer samples and 70 non-cancerous prostate samples were screened by the ARMS assay to detect the presence of R462Q polymorphism in RNASEL. Most of the patients with cancer were 70-79 years old (mean age: 70.88) and patients with non-cancerous prostate were 60-69 years old (mean age: 64.83). The incidence of RR (wild-type), RQ (heterozygous) and QQ (homozygous) variants presence in cancerous samples were 42 (82%), 7 (14%) and 2 (4%), respectively and 61 (87%), 9 (13%) and 0 (0%) in non-cancerous samples, respectively. The allelic frequency for R462Q in cancerous and non-cancerous patients was determined to be 0.108 and 0.064, respectively. It is the same as the results of a study by Shea et al. (12) and lower in comparison to other prostate cancer studies (the allel frequency of R462Q, 0.25

Table 1. Lists of the Used DNA Primer Sequences

Gene	Product Size	Primer Sequence	Primer Name
RNASEL	123 bp	5'-GTGGAAAATGAGGAAGATGAATTTGCCAG-3'	1385G
		5'-GTGGAAAATGAGGAAGATGAATTTGCCAA-3'	1385A
		5'-ATTGGGGACTCACCTATTAAGATGTTTTG-3'	1385R
Control gene	393 bp	5'-CCCACCTTCCCCTCTCTCCAGGCAAATGGG-3'	ARMS-A
		5'-GGGCCTCAGTCCCAACATAGGCTAAGAGGTG-3'	ARMS-B

Table 2. Association Between RNASEL Arg462Gln Polymorphism and Prostate Cancer in Patients With and Without Cancer [a, b]

Genotype	Cancerous Samples (n = 51)	Non-Cancerous Samples (n = 70)
Age, y (mean)	70-79 (70.88)	64-83 (64.83)
RR[c]	42 (82)	61 (87)
RQ[c]	7 (14)	9 (13)
QQ[c]	2 (4)	0 (0)
RR + RQ	49 (96)	121 (100)
R allele	91 (89.2)	131 (93.5)
Q allele	11 (10.8)	9 (6.5)
Allelic frequency for R462Q	0.108	0.064

[a] Abbreviations: QQ, arginine-argininee; RQ, glutamine-arginine; RR, glutamine-glutamin.
[b] Data are presented as No. (%).
[c] RR, wild-type; RQ, heterozygous; QQ, homozygous.

and 0.38) (9, 10, 13, 14). Prostate cancer was not found to be significantly associated with the R462Q polymorphism of RNASEL (P > 5% Fisher's exact test) (Table 2). Odds ratio was less than one, which means that this mutation was not related to prostate cancer.

5. Discussion

The third most common cancers among male population in Iran is prostate cancer (6). One factor with a probable role in the etiology of prostate cancer is chronic inflammation in prostate (3). Epidemiological studies indicated that prostatitis and sexually transmitted infections increase the risk of prostate cancer, while anti-inflammatory agents like aspirin decrease the risk of this disease (3). Some studies show relation of the 462Q/Q RNASEL genotype with viral infections and cancers of prostate (15). Studies of Casey et al. demonstrated reasonable evidence for the existence of prostate cancer susceptibility gene at the 1 q24-25 (11). In their study, a common polymorphism, namely Arg462Gln, was found to be associated with hereditary prostate cancer The studied RNASEL variant had three times less enzymatic activity than the wild type (11). An endoribonuclease, a member of the interferon regulated 2-5A system, is encoded by this gene. Some studies suggest that the RNASEL gene may function as a tumor suppressor gene, although some similar studies are not in agreement with this hypothesis (16). We, in our study investigated the relationship between RNASEL Arg462Gln gene polymorphism and sporadic prostate cancer risk; however a small proportion of cases with family prostate cancer history were included, because it is not feasible for us to separate the familial cases from the sporadic cases based on the available data (recorded information was defective). In this investigation no association was found between the RNASEL Arg462Gln polymorphism and prostate cancer. Review of some published linkage studies, including a nested case-control study in the USA (3), a population-based study conducted in Sweden (8), a meta-analysis study by Wei et al. (17), a German population study (18), a study of prostate cancer in Poland (19) and an analysis of the RNASEL in familial and sporadic prostate cancers by Wang et al. (5) did not show any association between the Arg462Gln polymorphism and sporadic prostate cancer risk. In a study by Meyer et al. mutation R462Q was only associated with an increased risk in the pre-prostate-specific antigen era and they suggested that this association may be mediated through inflammation (20). Larson et al. found that prostate cancer in patients with the R462Q mutation is not associated with more aggressive pathological or clinical features (21). Surprisingly, in an Asian study they found a decreased risk for familial prostate cancer in a Japanese population with the genotype Gln/Gln (22).

Kruger et al. investigated a variation at codon 462 of RNASEL in hereditary non-polyposis colorectal cancer. In their study the frequency of these codons did not differ between patients and controls; while sequence variation at RNASEL codon 462 was associated with age of onset in patients with cancer in a dose-dependent way (23). One of the reasons that we did not find any association between the Arg462Gln polymorphism and sporadic prostate cancer was the number of our samples. This is a pilot study and further studies are suggested with larger sample sizes. In other studies, like a study by Casey et al. (11) and the study of Alvarez-Cubero et al. in Spain (24), an association was found between this polymorphism and the sporadic prostate cancer risk. In the study of Casey et al. this relationship was also found among Caucasians and African Americans. The strongest the relationship between RNASEL and prostate cancer has been proven to be with the hereditary and familial prostate cancers and not with the sporadic forms (5, 11). The study by Robbins et al. showed RNASEL may play a role, however minor, in prostate cancer risk among African American men (25). To sum up, in the studied population, we found two cases with Gln/Gln genotype of codon 462. These cases were related to patients with prostate cancer, but these results were not statistically significant. Therefore, it is suggested that the Arg462Gln polymorphism in RNASEL does not play a significant role in the etiology of prostate cancer, in the population studied. However additional studies are essential to confirm the consequences of the RNASEL gene presence on the prostate cancer.

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Authors' Contributions

Seidabadi was in charge of the study, collecting data, performing the statistical analysis and preparing the manuscript. Rezatofighi supervised the study and participated in designing and conducting the study

and also manuscript preparation. Motamedi was a counselor of the study where he participated in the design of the study, the statistical analysis and helped in writing the manuscript. Rashidi was a counselor of the study where she participated in the collection and histopathologically detection of samples. All authors have studied and approved the content of the present manuscript.

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