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# Xenotropic Murine Leukemia Virus-Related Virus and RNase L R462Q Variants in Iranian Patients With Sporadic Prostate Cancer

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**Abstract**

**Background:** Although several studies have confirmed the association of xenotropic murine leukemia virus-related virus (XMRV) and prostate cancer, this association is still controversial, as most studies did not detect XMRV in prostate tissue samples. Furthermore, some genetic and epidemiological studies have highlighted a role for RNase L polymorphisms, particularly R462Q, in the progression of prostate cancer.

**Objectives:** The focus of this study was on the association of XMRV and RNase L R462Q variants with the risk of prostate cancer in Iranian patients.

**Patients and Methods:** In this case-control study, 40 and 80 individuals with sporadic prostate cancer and benign prostatic hyperplasia, respectively, were included. The presence of XMRV was evaluated by real-time polymerase chain reaction (PCR) of integrase and nested-PCR for the gag genes. The RNase L R462Q polymorphism analysis was carried out by PCR and sequencing.

**Results:** In a total of 40 sporadic prostate cancer and 80 benign prostatic hyperplasia cases, no XMRV was detected by real-time PCR and nested-PCR. RNase L R462Q polymorphism analysis reveals that although there was an increase in the risk of prostate cancer correlated with the Q/Q allele of RNase L at position 462, the frequencies of the RNase L R462Q alleles were not statistically significant between the prostate cancer and benign prostatic hyperplasia groups (OR = 2.75 (95% CI = 0.67 - 11.3), P = 0.29).

**Conclusions:** These results did not support the presence of XMRV in the samples with prostate cancer and showed that RNase L R462Q variants had relatively little or no impact on the risk of prostate cancer in Iranian population.

*Keywords:* Xenotropic Murine Leukemia Virus-Related Virus, Polymorphism, RNase L, Prostate Cancer

## 1. Background

Prostate cancer is the second most common cancer in males worldwide, affecting around 900 000 new cases annually. 258 400 deaths have been reported only in 2008 (1, 2). According to the reports by the International Agency for Research on Cancer, prostate cancer is the second most frequent cancer in Iranian males. In this regard, the age-standardized incidence rate of prostate cancer in Iran was estimated to be 11.6 (3).

The complexities of prostate cancer etiology harden the study of the affected subjects; however, both host genetics and environmental factors seem to play important roles in the development of prostate cancer (4-9). Genetic and epidemiologic studies supported the association of several RNase L mutations with prostate cancer, highlighting the role of RNase L in prostate cancer (7, 10-12). However, this association was not detected in all cases (13-15), indicating that either demographic diversities or environmental factors such as infections may play an important role in the development of prostate cancer.

A common missense mutation of RNase L at nucleotide 1385 (G1385A), which leads to an amino acid substitution of arginine to glutamine (R462Q), has been associated with prostate cancer (16, 17). The risk of prostate cancer increased in individuals who were homozygous for the Q allele (17). Indeed, a three-fold decrease in the catalytic activity of RNase L was reported in individuals carrying "Q" variant compared to the wild-type enzyme (7, 18).

Alongside with the host role, epidemiologic studies further supported the role of infection and inflammation in the development of prostate cancer (5, 19). In 2006, Uris-

man et al. reported the presence of a novel gammaretrovirus called xenotropic murine leukemia virus related-virus (XMRV) in some tissue samples taken from patients with prostate cancer (19). In addition, an important association between XMRV and the R462Q mutant of the RNase L gene was reported by authors, suggesting this virus to be a potential candidate human tumor virus in prostate cancer (19), as reported in some studies (20-22). Although not replicated by others, XMRV was detected in only a small subset of prostate tissue samples (23-27).

## 2. Objectives

To elucidate whether XMRV and RNase L R462Q variants play important roles in prostate cancer progression, we investigated the presence of XMRV and RNase L R462Q variants in Iranian patients with sporadic prostate cancer and benign prostatic hyperplasia.

## 3. Patients and Methods

### 3.1. Study Population and Samples

Formalin-fixed paraffin-embedded tissue samples of 40 sporadic prostate cancer (PCa) cases and 80 control tissues of benign prostatic hyperplasia (BPH) were included in this case-control study. All the samples were obtained from the participants in the Pathology Department of Sina Hospital, an urology referral hospital, in Tehran, Iran, during 2011. None of the patients had been treated by chemotherapy or radiotherapy up to the time of surgery. All the cases were re-examined to confirm the diagnosis. In total, 120 samples were tested for the presence of XMRV and analysis of the R462Q polymorphism in the RNase L gene.

### 3.2. DNA Extraction

For each paraffin block, four 10-μm sections were cut. Median sections were used for polymerase chain reaction (PCR) analysis. The first and final sections were applied for hematoxylin-eosin staining and were re-examined by the pathologist to confirm a Gleason score higher than 6. To prevent potential contamination of samples, the first four sections from each block were discarded, the microtome was cleaned with 70% ethanol and the blades were changed before cutting the other sample. In addition, an empty block was sliced between the blocks and used as a PCR control to check the potential cross-contamination through microtome use.

Genomic DNA was extracted according to previous published procedures with some modifications (28, 29). Briefly, two 10-μm sections for each tissue sample were extracted twice with 1 mL of xylenes, and twice with 0.5 mL of 100% ethanol for paraffin and organic solvents removal, respectively. The tissue samples were digested with lysis buffer (50 mM Tris-HCl pH 8.5, 1 mM EDTA, 0.5% Tween 20) containing 200 μg per mL proteinase K at 37°C overnight, followed by DNA purification by phenol-chloroform extraction and ethanol precipitation.

To assess the integrity of the DNA extracted from each tissue, a 268-bp segment of the β-globin gene was amplified. Each PCR reaction was performed in a 50-μL reaction mixture, including 2.5 mM $MgCl_2$, 20 pmol of each primer, 50 μM of each dNTP, 1.25 U of Taq DNA polymerase, and 100 - 200 ng of the DNA template. The PCR was performed as follows: an initial five-minute denaturation at 94°C, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final elongation at 72°C for five minutes (30). Following DNA integrity assay all the samples were appropriate for analysis.

### 3.3. Amplification of Xenotropic Murine Leukemia Virus-Related Virus

The list of primers and the probe used in this study is shown in Table 1. A 122-bp region of the integrase gene of XMRV that is completely conserved between the variants was selected to detect the proviral DNA of XMRV by real-time PCR. The amplification reaction was carried out in a 30-μL reaction mixture containing TaqMan Fast Universal PCR Master Mix (Applied BioSystems, Foster City, USA), 20 pmol of each primer, 7 pmol of hydrolysis probe and 100-200 ng of the target DNA. The target gene was amplified in a StepOnePlus™ Real-Time PCR System (Life Technologies, USA) with the following steps: an initial two-minute denaturation cycle at 95°C followed by 50 cycles of 95°C for 15 seconds and 60°C for one minute. The plasmid DNA containing the target fragment was constructed (Metabion, Germany) and used as a positive control. In addition, a nested-PCR for the gag gene of XMRV was carried out according to the previous published protocol (19).

### 3.4. R462Q RNase L Polymorphism Analysis

The R462Q RNase L polymorphism analysis was carried out by PCR, followed by direct nucleotide sequencing. A 224-bp amplicon size of the RNase L containing the polymorphic site was amplified with the primer pair shown in Table 2. The PCR reactions were performed in a 50-μL reaction mixture including 2 mM $MgCl_2$, 20 pmol of each primer, 50 μM of each dNTP, 2 U of Taq DNA polymerase and 100 - 200 ng of the DNA template. The PCR was performed as follows: an initial three-minute denaturation at 95°C, followed by 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds, 72°C for one minute and a final elongation at 72°C for five minutes.

The PCR products were sequenced directly using BigDye® Terminator version 3.1 Cycle Sequencing Kit and a 3130 Genetic Analyzer Automated Sequencer, as specified by the Applied BioSystems protocols (Applied BioSystems, Foster City, CA). The nucleotide sequences were edited with BioEdit program.

### 3.5. Statistical Analysis

The observed R462Q RNase L genotype frequencies for the PCa and BPH groups were assessed by the Hardy-Wein-

berg equilibrium theory. To compare the proportions of R462Q RNase L alleles between the PCa and BPH groups, a Fisher's exact test or $\chi^2$ test and to compare differences between the mean ages of the PCa and BPH groups, a t-test were carried out by Epi Info 7. P values less than 0.05 were considered statistically significant.

## 4. Results

The present study included a total of 120 tissue samples from 40 PCa and 80 BPH subjects. The demographic and pathologic features of PCa and BPH cases are shown in Table 3. The median age at the diagnosis was 71 years (mean: 69.85 ± 8.58) for PCa and 70 years (mean: 69.91 ± 8.05) for BPH (P > 0.64).

The integrity of genomic extracts was confirmed by PCR of the β-globin gene for all the 120 samples. However, all the 40 PCa and 80 BPH patients were negative for the detection of the XMRV genome.

The R462Q RNase L polymorphism analysis was carried out using PCR followed by direct nucleotide sequencing (Figure 1). The frequency of R462Q RNase L genotypes in PCa and BPH cases are summarized in Table 3. The R462Q RNase L allele frequencies were found in Hardy-Weinberg equilibrium among the PCa and BPH groups ($\chi^2$ = 0.65; df = 1; P > 0.05 and $\chi^2$ = 0.36; df = 1; P > 0.05, respectively).

The frequencies of the R462Q RNase L R/R, R/Q and Q/Q alleles among the 40 PCa cases were 50% (n = 20), 37.5% (n = 15) and 12.5 % (n = 5), respectively, and among the 80 BPH subjects they were 55% (n = 44), 40% (n = 32) and 5% (n = 4), respectively. Crude odds ratios (OR) and 95% confidence intervals (CI) were used to evaluate the correlation between the R462Q RNase L genotypes and the risk of prostate cancer (Table 2).

As shown in Table 2, there was an increase in the risk of prostate cancer correlated with the Q/Q allele of RNase L at position 462 in comparison with the R/R allele (OR = 2.75, 95% CI = 0.63 - 11.3). However, the frequencies of the RNase L R462Q alleles were not statistically significant between the PCa and BPH groups (P > 0.05).

The stratification of PCa and BPH groups was performed regarding to the age and Gleason score. There was no important association between age and the Gleason score and RNase L R462Q genotypes in the PCa and BPH groups (Tables 4 and 5).

**Table 1.** List of Primers and the Probe Used in This Study

| Locus | Primer and Probe Name | Sequence | Amplicon Size, bp | References |
|---|---|---|---|---|
| **XMRV** | | | 122 | (22) |
| | XMRV4552F | 5'-CGAGAGGCAGCCATGAAGG-3' | | |
| | XMRV4673R | 5'-CCC AGT TCC CGT AGT CTT TTG AG-3' | | |
| | XMRV4572MGB | 5'-6FAMAGTTCTAGAAACCTCTACACTCMGBNFQ-3' | | |
| **XMRV** | | | 612 | (19) |
| | GAG-O-F | 5'-CGCGTCTGATTTGTTTTGTT-3' | | |
| | GAG-O-R | 5'-CCGCCTCTTCTTCATTGTTC-3' | | |
| | GAG-I-F | 5'-TCTCGAGATCATGGGACAGA-3' | 413 | |
| | GAG-I-R | 5'-AGAGGGTAAGGGCAGGGTAA-3' | | |
| **RNase L** | | | 224 | (13) |
| | Arg462Gln-F | 5-AAGCAGCCGAGAGAACAGTC'-3' | | |
| | Arg462Gln-R | 5'-GGTGGGTGTATCCACAGGAC-3' | | |
| B **Globin** | | | 268 | (30) |
| | GH20 | 5'-GAAGAGCCAAGGACAGGTAC-3' | | |
| | PC04 | 5'-CAACTTCATCCACGTTCACC-3' | | |

**Table 2.** Distribution of RNase L R462Q Variants in Sporadic Prostate Cancer (PCa) and Benign Prostatic Hyperplasia (BPH) Cases[a]

| Genotype[b] | PCa, (n = 40)[c] | BPH, (n = 80)[c] | OR (95% CI) | P Value |
|---|---|---|---|---|
| RR | 20 (50) | 44 (55) | 1 | |
| RQ | 15 (37.5) | 32 (40) | 1.031 (0.46 - 2.3) | 0.89 |
| QQ | 5 (12.5) | 4 (5) | 2.75 (0.67 - 11.3) | 0.29 |
| **Recessive model** | | | | |
| RR + RQ | 35 (87.5) | 76 (95) | 1 | |
| QQ | 5 (12.5) | 4 (5) | 2.7 (0.69 - 10.7) | 0.27 |
| **Dominant model** | | | | |
| RR | 20 (50) | 44 (55) | 1 | |
| RQ + QQ | 20 (50) | 36 (45) | 1.2 (0.57 - 2.6) | 0.75 |

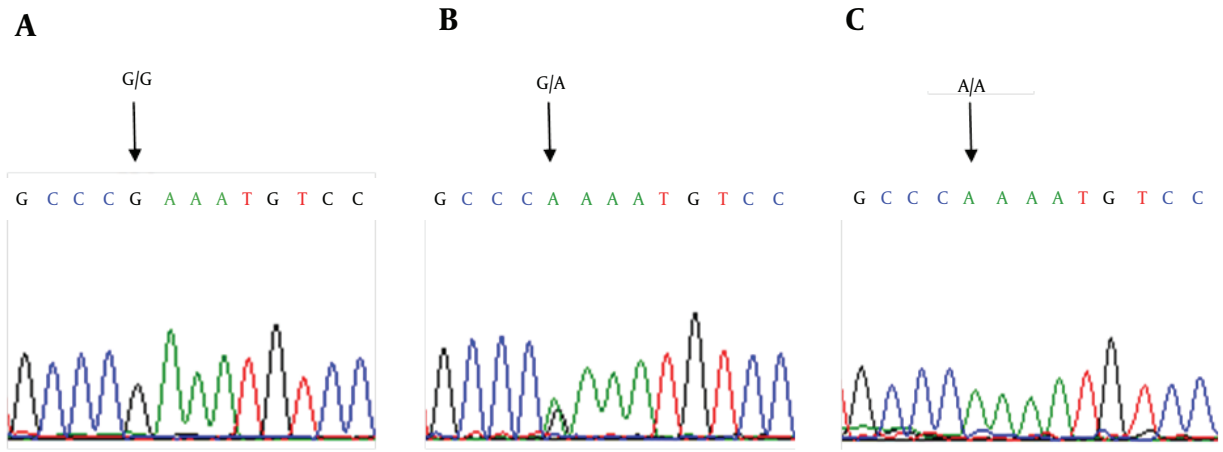
[a]Abbreviations: BPH, benign prostatic hyperplasia; PCa, sporadic prostate cancer.
[b]Alleles: RR = Arginine/Arginine, QQ = Glutamine/Glutamine, RQ = Arginine/Glutamine.
[c]Values are presented as No. (%).

**Table 3.** The Demographic and Pathologic Features of Sporadic Prostate Cancer (PCa) and Benign Prostatic Hyperplasia (BPH) Cases[a]

| Characteristics | Number of Subjects[b] | RNase L R462Q Alleles[c] | | |
|---|---|---|---|---|
| | | RR | RQ | QQ |
| **Prostate Cancer** | | | | |
| N | 40 | 20 (50) | 15 (37.5) | 5 (12.5) |
| Age, y | | | | |
| ≤ 70 | 16 | 8 (50) | 6 (37.5) | 2 (12.5) |
| > 70 | 24 | 12 (50) | 9 (37.5) | 3 (12.5) |
| **Gleason score** | | | | |
| 6 - 7 | 12 (30) | 7 (58.4) | 4 (33.3) | 1 (8.3) |
| 8 - 10 | 28 (70) | 13 (46.4) | 11 (39.3) | 4 (14.3) |
| **Benign prostatic hyperplasia** | | | | |
| N | 80 | 44 (55) | 32 (40) | 4 (5) |
| Age, y | | | | |
| ≤ 70 | 33 | 20 (60.6) | 11 (33.3) | 2 (6.1) |
| > 70 | 47 | 24 (51.1) | 21 (44.7) | 2 (4.2) |

[a]Alleles: RR = Arginine/Arginine, RQ = Arginine/Glutamine, QQ = Glutamine/Glutamine.
[b]Values are presented as No. (%). or %.
[c]Values are presented as No. (%).

**Figure 1.** Nucleotide Sequencing Results of the Polymerase Chain Reaction product of RNase L Gene




a) Common homozygote R/R; b) heterozygote R/Q; c) rare homozygote Q/Q.

**Table 4.** Distribution of RNase L R462Q Variants in Sporadic Prostate Cancer and Benign Prostatic Hyperplasia Cases, Stratified By Age[a,b]

| Age, y | ≤ 70 | | | > 70 | | |
|---|---|---|---|---|---|---|
| | PCa, (n = 16)[c] | BPH, (n = 33)[c] | (95% CI) | PCa, (n = 24)[c] | BPH, (n = 47)[c] | OR (95% CI) |
| **Genotype** | | | | | | |
| RR | 8 (50) | 20 (60.6) | 1 | 12 (50) | 24 (51.1) | 1 |
| RQ | 6 (37.5) | 11 (33.3) | 1.36 (0.37 - 4.9) | 9 (37.5) | 21 (44.7) | 0.85 (0.3 - 2.4) |
| QQ | 2 (12.5) | 2 (6.1) | 2.5 (0.3 - 20.9) | 3 (12.5) | 2 (4.2) | 3 (0.4 - 20.4) |
| **Recessive model** | | | | | | |
| RR + RQ | 14 (87.5) | 31 (93.9) | 1 | 21(87.5) | 45 (95.8) | 1 |
| QQ | 2 (12.5) | 2 (6.1) | 2.2 (0.3 - 17.3) | 3 (12.5) | 2 (4.2) | 3.2 (0.5 - 20.7) |
| **Dominant model** | | | | | | |
| RR | 8 (50) | 20 (60.6) | 1 | 12 (50) | 24 (51.1) | 1 |
| RQ + QQ | 8 (50) | 13 (39.4) | 1.5 (0.46 - 5.1) | 12 (50) | 23 (48.9) | 1.04 (0.4 - 2.7) |

[a]Abbreviations: BPH, benign prostatic hyperplasia, PCa, sporadic prostate cancer.
[b]Alleles: RR = Arginine/Arginine, RQ = Arginine/Glutamine, QQ = Glutamine/Glutamine.
[c]Values are presented as No. (%).

**Table 5.** Distribution of RNase L R462Q Variants in Sporadic Prostate Cancer Stratified By Pathological Grade[a]

| | Gleason Score 6 - 7,[b] | Gleason Score 8 - 10,[b] | OR (95% CI) | P Value |
|---|---|---|---|---|
| **Genotype** | | | | |
| RR | 7 (58.4) | 13 (46.4) | 1 | |
| RQ | 4 (33.3) | 11 (39.3) | 0.67 (0.15 - 2.9) | 0.87 |
| QQ | 1 (8.3) | 4 (14.3) | 0.46 (0.04 - 5) | 0.91 |
| **Recessive model** | | | | |
| RR + RQ | 11 (91.7) | 24 (85.7) | 1 | |
| QQ | 1 (8.3) | 4 (14.3) | 0.54 (0.05 - 5.4) | 0.99 |
| **Dominant model** | | | | |
| RR | 7 (58.4) | 13 (46.4) | 1 | |
| RQ + QQ | 5 (41.6) | 15 (53.6) | 0.62 (0.16 - 2.4) | 0.73 |

[a]Alleles: RR = Arginine/Arginine, RQ = Arginine/Glutamine, QQ = Glutamine/Glutamine.
[b]Values are presented as No. (%).

## 5. Discussion

Previous linkage analyses on prostate cancer showed that the HPC1 locus likely harbors a prostate cancer susceptibility gene (12, 14). RNase L was first proposed as a candidate gene for this region by Carpten et al. in 2002 (10). Moreover, Urisman et al. reported the presence of XMRV in prostate cancer tissues and found an association between XMRV and the R462Q mutant of the RNase L gene (19).

To investigate whether XMRV is present in tissues obtained from prostate cancer and the RNase L R462Q variants are associated with the risk of prostate cancer, 40 sporadic PCa and 80 BPH cases were analyzed using both real-time PCR of integrase and nested-PCR for the gag genes. In agreement with recent studies that also showed general absence of XMRV or detection of it in a small subset of cases (23-27), XMRV was not detected in our study groups, suggesting that the distribution of XMRV may vary in different geographic regions (24). In a study in Kerman, Iran, XMRV was detected only in 4% of patients with prostate cancer (31). The lack of XMRV in the present study may be due to population heterogeneity, as these patients referred to Sina Hospital from all over the country. Unlike our study subjects where patients and healthy groups were heterogeneous, the study population of Kerman was homogenous. Future investigations need to determine the geographical dependence of XMRV occurrence in different parts of Iran. Alternatively, it has been suggested that XMRV may not be a genuine human pathogen, as XMRV detected by sensitive PCR assays in prostate cancer samples may be a consequence of PCR contamination with mouse DNA (32-34).

In the present study, RNase L R462Q polymorphism analysis revealed that the frequencies of the RNase L R462Q alleles were not statistically significant, although the rate of Q/Q allele of RNase L in PCa was higher than the control group. Our study showed a weak support of RNase L as a prostate cancer susceptibility gene. The stratification of the PCa and BPH groups according to age revealed that there was no significant association between age and RNase L R462Q genotypes in the PCa and BPH groups. Association analysis of RNase L R462Q genotypes in prostate cancer is controversial. Although some studies reported an association between this variant and the risk of prostate cancer (7, 12), others could not find such association (14-16, 35). In a meta-analysis performed on 3009 patients with prostate cancer and 703 familial prostate subjects, no significant association was found between RNase L R462Q variants and risk of prostate cancer (OR = 1.04, 95% CI = 0.68 - 1.60 and OR = 1.67, 95% CI = 0.69 - 4.08, respectively) (36)

In conclusion, the results of this study supported neither association between the age nor the Gleason score and RNase L R462Q variants. Our results also did not support the presence of XMRV in prostate cancer samples so far. In addition, RNase L R462Q variants seem to have relatively small or no impact on the risk of prostate cancer in the Iranian population. However, the small sample size was a clear limitation of the present study. To confirm the lack of XMRV in this cancer and the role of this RNase L polymorphism in prostate cancer progression, the results need to be verified by further studies with larger sample sizes in different parts of the country.

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## Footnotes

**Authors' Contribution:**Farhad Babaei, Ali Ahmad, Rakhshande Nategh and Talat Mokhtari Azad contributed to design of the study; Farhad Rezaei, Nastaran Ghavami, Mahmoud Mahmoudi and Nasim Kondori contributed to analysis and interpretation of data; Somayeh Jalilvand and Ramin Abiri contributed to drafting of the article.

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