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Research Article

# Association of *HNF1B*-rs4794758 and *LMTK2*-rs7791463 Gene Variants with Prostate Neoplasia Risk: Case-control and Bioinformatics Studies

Fereshteh Jozaghkar[1], Abasalt Hosseinzadeh Colagar[1*], Mohammadreza Hosseinzadeh[1], Faramarz Mehrnejad[2], Emadoddin Moudi[3]

[1] *Department of Molecular and Cell Biology, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran*
[2] *Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran*
[3] *Clinical Research Development Unit of Shahid Beheshti Hospital, Babol University of Medical Sciences, Babol, Mazandaran, Iran*

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## Abstract

Prostate neoplasms, such as prostate cancer and benign prostatic hyperplasia, are complex and heterogeneous diseases that are caused by environmental, metabolic, and genetic factors. Various reports showed the relationship of several genes, including the *HNF1B* and *LMTK2* genes, in the occurrence of prostate cancer. This study investigated the association of *HNF1B*-rs4794758 and *LMTK2*-rs7791463 polymorphisms with prostate cancer and benign prostatic hyperplasia in a case-control study, followed by bioinformatics analysis. For this purpose, blood samples were collected from 70 healthy men, 58 men with benign prostatic hyperplasia (positive digital rectal examination or DRE and PSA levels below 4 ng/mL), and 70 men with prostate cancer (positive DRE, PSA levels above 4 ng/mL, and diagnoses confirmed by pathological findings). These men were referred to Shahid Beheshti Hospital in Babol. After genomic DNA extraction, the genotype was determined using PCR-RFLP. A genotypic and allelic analysis revealed that the rs4794758 polymorphism with AA genotype (OR: 4.808, 95%CI: 1.260-18.348, P= 0.022) had a significant difference between the prostate cancer group and the benign prostatic hyperplasia group compared to the control group. Allele A of this polymorphism was also significantly associated with prostate cancer (OR: 1.705, 95%CI: 1.055-2.755, P= 0.030). However, there was no correlation between different genotypes of the rs7791463 polymorphism with prostate cancer and benign prostatic hyperplasia. Bioinformatics analysis by some online servers and software showed that the rs4794758 polymorphism possibly changes the hnRNA splicing pattern. So, this polymorphism could probably provide a locus for the TBP transcription factor. In addition, the rs7791463 polymorphism potentially alters the hnRNA splicing pattern and changes the reading frame. Based on the data, *HNF1B*-rs4794758 polymorphism is associated with prostate cancer susceptibility, which can be considered a molecular risk factor in future studies.

***Keywords*:** Benign prostate hyperplasia; Genetic polymorphism; *HNF1B* gene; *LMTK2* gene; Prostate cancer

## Introduction

The prostate gland, one of the most critical accessory glands in the male reproductive system, can be affected by prostatitis, benign prostatic hyperplasia (BPH), and prostate cancer (Kumar and Majumder, 1995). BPH affects half of men over 40 and 90% after 80, while prostate cancer is the second most common cancer in men worldwide. Cancer cells are found in 80% of men's prostate glands by age 80 (Denmeade and Isaacs, 2004; Kim et al., 2016). Many single nucleotide polymorphisms (SNPs) introduced by GWAS are located in regions of the genome that are not translated into protein (Colagar et al., 2023). These SNPs exert their effect through epigenetics, histone modifications, or action at the mRNA level. Genetic studies have identified susceptible variants for prostate cancer, such as the rs4794758 polymorphism in the *HNF1B* gene and the rs7791463 polymorphism in the *LMTK2* gene (Yeager et al., 2008). These variants may increase or decrease prostate gland disorder risk. Hepatocyte nuclear factor 1 beta, encoded by the *HNF1B*, regulates various organs. Prostate cancer progression enhances mRNA and nuclear HNF1B protein expression, while cytoplasmic *HNF1B* expression declines (Igarashi et al., 2005; Harries et al., 2010). The *LMTK2*, a transmembrane serine/tyrosine kinase, increases prostate cell proliferation when its activity is decreased (Puri et al., 2010). This study examines the association between rs4794758 and rs7791463 polymorphisms

* Corresponding author's e-mail address: *ahcolagar@umz.ac.ir, acolagar@yahoo.com*

with prostate cancer and benign hyperplasia risk in the population of Babol City, Mazandaran, Iran.

## Materials and Methods

### Study participants and sample collection

This case-control study involved 70 prostate cancer, 58 BPH, and 70 healthy men who were referred to Shahid Beheshti Hospital in Babol, Mazandaran, Iran. The control group consisted of asymptomatic individuals undergoing routine annual check-ups. Participants were selected based on the absence of prostate-related symptoms and a serum PSA level below 4 ng/mL, as documented in their medical history. Based on symptoms and medical records, the BPH and cancer groups were selected from individuals who had undergone surgery following a definitive diagnosis by a urologist. The BPH group specifically included individuals with a positive digital rectal examination or DRE (categorized as negative for ≤3 and positive for >3) and a PSA level below 4 ng/mL. The prostate cancer group consisted of individuals with a positive DRE, PSA levels above 4 ng/mL, and a diagnosis confirmed by pathological findings. About 2 ml of blood with sodium citrate anticoagulant was prepared and stored at -20°C for further use. This study was approved by the Ethics Committees of the Mazandaran University of Medical Science (#IR.UMZ.REC.1397.056) and all subjects signed an informed consent form before entering the study.

### DNA extraction and genotyping of samples

Genomic DNA was extracted from blood samples using phenol-chloroform, and polymorphisms rs4794758 and rs7791463 were genotyped via PCR-RFLP. *HNF1B* (NC_000017) and *LMTK2* (NC_000007) sequences were obtained from the NCBI database. Oligo7 software (MBI, USA) was used to design specific primers based on the sequence. Forward and reverse oligonucleotide primers (Table 1) were purchased from Bioneer Co. (South Korea) and Tekaposist Co. (Iran). The PCR reaction was performed in a thermal cycler (Eppendorf Co., Germany), and the resulting PCR products were loaded onto an agarose gel for quality verification.

The *Rsa*I and *Nco*I restriction enzymes (Fermentas; Burlington, Canada) were used to treat PCR products with rs4794758 and rs7791463 polymorphisms. Two samples with different genotypes were sent to Pishgam Biotechnology Co. (Iran) for verification. Chromas sequence chromatogram viewer software (http://www.technelysium.com.au/chromas_lite.html) was used for extraction and analysis, while the BLAST program (http://www.ncbi.nlm.nih.gov) searched and aligned the extracted sequences.

### Bioinformatics analysis

The RNAsnp server (www.rth.dk/resources/rnasnp/) analyzes the effects of the mentioned polymorphisms on the mRNA secondary structure. The hnRNA splicing was performed using the Human Splicing Finder (http://www.umd.be/HSF3/HSF.shtml) and ASSP servers (http://wangcomputing.com/assp/index.html). AliBaba v2.1 (http://gene-regulation.com/pub/programs/alibaba2) examines the effects of polymorphism transcription factor binding site. Fathmm (http://fathmm.biocompute.org.uk), Mutation Tasting (http://www.mutationtaster.org), and RegulationSpotter (https://www.mutationdistiller.org/RegulationSpotter/AnalyseVariant.html) explore the general effects of the mentioned polymorphisms. A Polyphen-2 server examines non-synonymous SNP effects on protein structure and function (http://genetics.bwh.harvard.edu/pp2).

**Table 1.** Characteristics of specific primers and amplification program of DNA fragments using PCR technique

| Gene& SNP ID | Primer name: Oligomer (5'→3') | PCR conditions | PCR products |
|---|---|---|---|
| *HNF1B* (intron4) rs4794758 | F: 5'-GCACATGGTAGACACTCC<br>R: 5'-AAACAAAAGAGGAGACGTTC | Initial denaturation: 95°C/5 min.; 35 Cycles (94°C/30s., 55.5°C/45s.,72°C/45s); and Final extension: 72°C/5 min. | 472-bp |
| *LMTK2* (intron9) rs7791463 | F: 5'-ACACAAATGCGGGATGGAGG<br>R:5'-CGTGGGTTTTGCTGCTATTCTG | Initial denaturation: 95°C/5 min.; 35 Cycles (95°C/30s., 55.1°C/45s.,72°C/30s); and Final extension: 72°C/5 min. | 257-bp |

PROVEAN (http://provean.jcvi.org/seq_submit.php) evaluates and predicts the impact of amino acid substitutions on proteins. DbPTM 3.0 (http://dbptm.mbc.nctu.edu.tw/index.php) identifies and introduces PTMs reported in practical experiments, solvent accessibility, secondary and tertiary proteins, and their variants. An interaction diagram was generated using the BioGRID database (http://thebiogrid.org/)

### Statistical analysis

Hardy-Weinberg equilibrium was checked for each SNP using a chi-square test for control, BHP, and prostate cancer samples. Based on the Binary Logistic Regression test, we investigated the relationship between genotypes and alleles and the risk of BHP and prostate cancer. For this purpose, the Odd Ratio (OR) and the 95% Confidence Interval (CI) were calculated for different genotypes and alleles. A P-value less than 0.05 ($P< 0.05$) was considered statistically significant. The statistical analysis was performed using SPSS 16.

## Results

### Genotyping of SNPs

The G allele at rs4794758 generates a recognition site for the *Rsa*I enzyme, which cuts the next nucleotide. On agarose gel, samples with the GG and AG genotype have two bands (214bp and 258bp and 472bp, 258bp, and 214bp), while the AA genotype shows only one band (472bp) (Figure 1A). However, if the G allele is located in the rs7791463 polymorphic position, the *Nco*I enzyme cuts the site. As a result, GG genotype samples have two bands (112bp and 145bp), GA genotype samples have three bands (257bp, 145bp, and 112bp), while AA genotype samples have one band (257bp) on agarose gel (Figure 1B). Direct sequencing of two homozygous samples confirmed PCR-RFLP results.

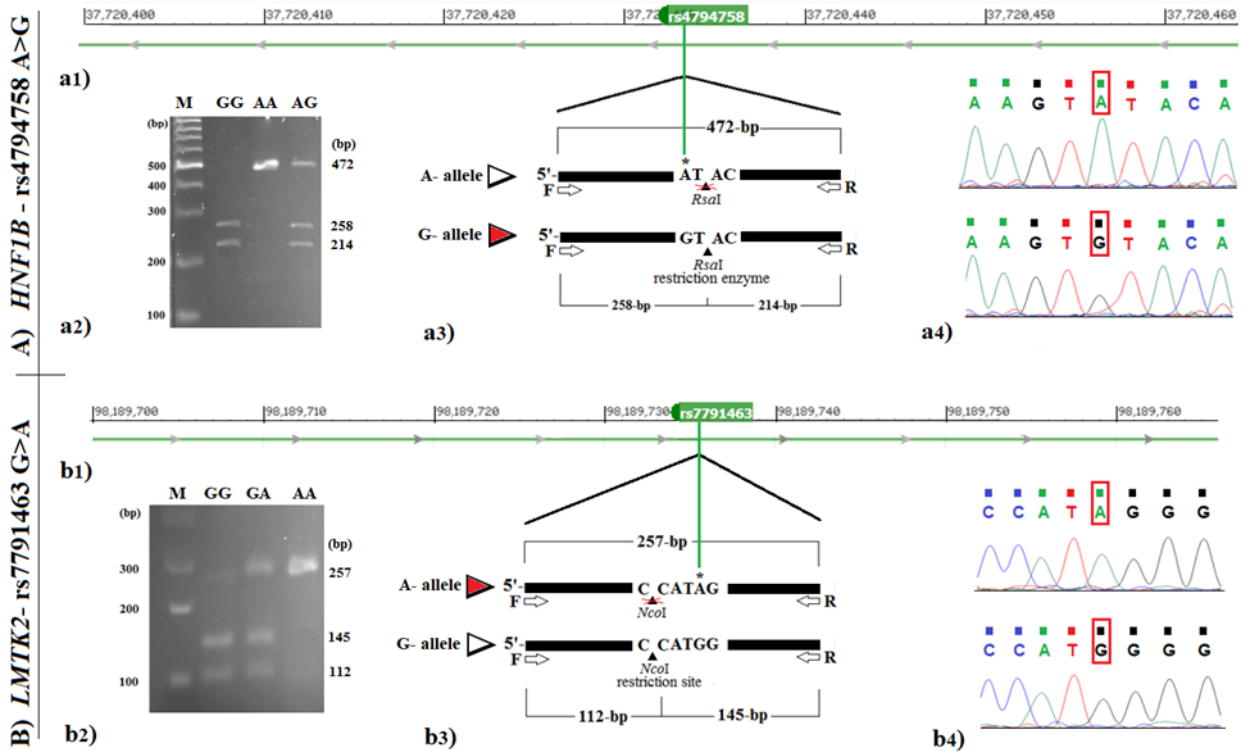



**Figure 1.** Schematic map and RFLP pattern of the PCR fragments: A) Schematic and restriction pattern of *HNF1B* - rs4794758 (A>G) fragments, **a1** shows rs4794758 position in the *HNF1B* gene, **a2** is RFLP pattern of the PCR fragments with three GG, AA, and AG genotypes, **a3** showed a schematic map of *Rsa*I restriction digestions and **a4** showed electropherograms of flanking nucleotides of this SNP; B) Schematic and restriction pattern of *LMTK2*- rs7791463 (G>A) fragments, **b1** showed rs7791463 position in the *LMTK2* gene, **b2** is RFLP pattern of the PCR fragments with three GG, AA, and GA genotypes, **b3** showed a schematic map of *Nco*I restriction digestions and **b4** showed electrophirograms of flanking nucleotides of this SNP; M showed DNA ladder.

**Distribution of allele and genotype frequencies**

The study found that the rs4794758 and rs7791463 polymorphism genotypes were in Hardy-Weinberg equilibrium in the control, BPH, and prostate cancer groups (Table 2). At the rs4794758 polymorphism, the AG and AA genotype frequencies in the control group were 67% and 18.6%, respectively. The BPH group had 58.6% and 32.8%, and the prostate cancer group had 58.6% and 35.7%. The allele frequencies for G and A were 47.86% and 52.14, respectively. The frequency of AG and AA genotypes in the BPH group was 37.99% and 62.07%, while in the prostate cancer group, it was 35% and 65%. In the BPH group, AG (OR: 1.447, 95%CI: 0.453-4.618, P= 0.533) and AA (OR: 2.923, 95%CI: 0.809-10.561, P= 0.102) genotypes were not significantly different from healthy individuals. The frequency of the AG genotype (OR: 2.181, 95%CI: 0.636-7.483, P= 0.215) in the prostate cancer group was not significantly different from the healthy group, but the frequency of the AA genotype (OR: 4.808, 95%CI: 1.260-18.348, P = 0.022) was significantly different. There were no significant differences between the prostate cancer and BPH groups regarding AG (OR: 1.507, 95%CI: 0.375-6.059, P= 0.563) or AA (OR: 1.645, 95%CI: 0.388-6.968, P= 0.499) genotypes. Carriers of the A allele (AG+AA) were not at high risk for BPH (OR: 1.767, 95%CI: 0.568-5.498, P= 0.326) and prostate cancer (OR: 2.750, 95%CI: 0.819-9.232, P= 0.102). Moreover, the A allele is not considered a risk factor for BPH (OR: 1.502, 95%CI: 0.910-2.478, P= 0.111), despite being considered for prostate cancer (OR: 1.705, 95%CI: 1.055-2.755, P= 0.030).

The AG and AA genotype frequencies of the rs7791463 polymorphism were 52.8% and 25.7% in the control group, respectively. The BPH group had frequencies of 61% and 26%, while the prostate cancer group had frequencies of 67.1% and 12.9%. The G and A allele frequencies were 47.9% and 52.1% in the control group, while the BPH group had frequencies of 44% and 56%, and 46.4% and 53.6%, respectively. Data analysis showed that the frequency of AG (OR: 1.937, 95%CI: 0.760-4.939, *P*= 0.166) and AA (OR: 1.667, 95%CI: 0.581-4.779, *P*= 0.342) genotypes in the BPH group and the frequency of AG (OR: 1.361, 95%CI: 0.584-3.172, *P*= 0.475) and AA (OR: 0.536, 95%CI: 0.182-1.581, *P*= 0.258) genotypes in the prostate cancer group were not significantly different compared to the healthy group. The AG genotype frequency (OR: 0.703, 95%CI: 0.276-1.788, P = 0.459) was not significantly different between prostate cancer and BPH. However, the frequency of the AA genotype (OR: 0.321, 95%CI: 0.101-1.024, *P*= 0.055) was different in the prostate cancer group compared to the BPH group, but this difference was not statistically significant. Carriers of the A allele (AG+AA) were not at high risk for BPH (OR: 1.848, 95%CI: 0.749-4.561, P= 0.182) or prostate cancer (OR: 1.091, 95%CI: 0.481-2.472, P= 0.835), and further analysis of allele frequencies revealed that the A allele was not associated with BPH (OR: 1.189, 95%CI: 0.742-1.903, P= 0.472) or prostate cancer (OR: 1.059, 95%CI: 0.662-1.693, P= 0.811).

***In silico* analysis of rs4794758 and rs7791463 transitions**

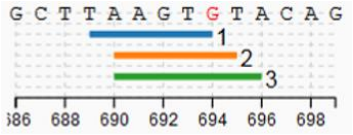
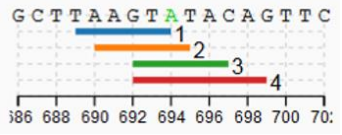
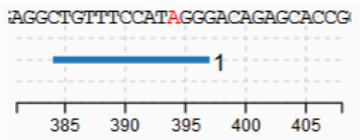
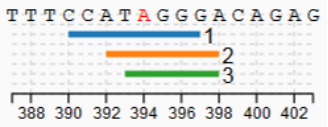
Based on biological data modeling techniques, different online software packages were used to analyze the *HNF1B*-rs4794758 transition. According to the Human Splicing Finder server results, the rs4794758 change causes the loss of four enhancers and two silencer motifs and creates three new silencer motifs (Table 3A).

According to the ASSP server and Alibaba v2.1 software, the rs4794758 transition does not affect mRNA splicing patterns (Supplementary files: Figure S1) but creates a binding site for the TBP transcription factor. The FATHMM v2.3 online software did not reveal a significant effect. The rs4794758 transition participates in muscle cell acetylation and tri-methylation, creating a DNase1-sensitive site in embryonic stem cells. The HNF1B protein interaction network in humans was analyzed using the BioGRID server (Supplementary files: Figure S2). RNAsnp database showed that the *LMTK2*-rs7791463 polymorphism did not significantly affect the mRNA structure (P= 0.7486; Figure 2). The Human Splicing Finder server reported that this polymorphism created a branch point motif, an acceptor site, four enhancer motifs, and two new silencer motifs. It also destroyed one exon splicing regulatory motif, one enhancer motif, and five silencer motifs (Table 3B). ASSP server results indicate three changes in the reading frame due to this polymorphism (Supplementary files: Figure S1). AliBaba ver. 2.1 and Fathmm ver. 2.3 online software did not show a significant effect. This polymorphism is involved in the tri-methylation of lysine 27 of histone 3 in blood cells and in the acetylation of lysine 27 of histone 3 in muscle cells, according to RegulationSpotter analysis. The LMTK2 protein interaction network is depicted in the BioGRID database (Supplementary files: Figure S2).

**Table 2.** Analysis of *HNF1B*-rs4794758 and *LMTK2*-rs7791463 gene variants with prostate hyperplasia (BPH and cancer).

| SNPs | Genotype / Allele | Number (Percentage) | | | Association of gene variants with BPH group | | | | | Association of gene variants with cancer group | | | | | Association of cancer gene variants compared to the BPH group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control N=70 | BPH N=58 | Cancer N=70 | OR | 95% CI Lower | 95% CI Upper | df | P-value | OR | 95% CI Lower | 95% CI Upper | df | P-value | OR | 95% CI Lower | 95% CI Upper | df | P-value |
| **A) HNF1B-rs4794758** | **GG** | 10 (14.4%) | 5 (8.6%) | 4 (5.7%) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **AG** | 47 (67%) | 34 (58.6%) | 41 (58.6%) | 1.447 | 0.453 | 4.618 | 1 | 0.533 | 2.181 | 0.636 | 7.483 | 1 | 0.215 | 1.507 | 0.375 | 6.059 | 1 | 0.563 |
| | **AA** | 13 (18.6%) | 19 (32.8%) | 25 (35.7%) | 2.923 | 0.809 | 10.561 | 1 | 0.102 | 4.808 | 1.260 | 18.348 | 1 | **0.022** | 1.645 | 0.388 | 6.968 | 1 | 0.499 |
| | **G** | 67 (47.86%) | 44 (37.93%) | 49 (35%) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **A** | 73 (52.14%) | 72 (62.07%) | 91 (65%) | 1.502 | 0.910 | 2.478 | 1 | 0.111 | 1.705 | 1.055 | 2.755 | 1 | **0.030** | 0.881 | 0.529 | 1.469 | 1 | 0.627 |
| **B) LMTK2-rs7791463** | - | **Control N=70** | **BPH N=70** | **Cancer N=70** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **GG** | 15 (22.5%) | 9 (13%) | 14 (20%) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **AG** | 37 (52.8%) | 43 (61%) | 47 (67.1%) | 1.937 | 0.760 | 4.939 | 1 | 0.166 | 1.361 | 0.584 | 3.172 | 1 | 0.475 | 0.703 | 0.276 | 1.788 | 1 | 0.459 |
| | **AA** | 18 (25.7%) | 18 (26%) | 9 (12.9%) | 1.667 | 0.581 | 4.779 | 1 | 0.342 | 0.536 | 0.182 | 1.581 | 1 | 0.258 | 0.321 | 0.101 | 1.024 | 1 | 0.055 |
| | **G** | 67 (47.9%) | 61 (44%) | 65 (46.4%) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **A** | 73 (52.1%) | 79 (56%) | 75 (53.6%) | 1.189 | 0.742 | 1.903 | 1 | 0.472 | 1.059 | 0.662 | 1.693 | 1 | 0.811 | 0.891 | 0.556 | 1.427 | 1 | 0.631 |

**Table 3.** Analysis of transition effects of the rs4794758 and rs7791463 on the mRNA transcripts of the *HNF1B* and *LMTK2* genes by HSF prediction tool.

| Predicted signal | Prediction algorithm | cDNA Position | Interpretation |
|---|---|---|---|
| **A) *HNF1B* -rs4794758 (A>G) *** | | | |
| New ESS Site | 1- Fas-ESS hexamers<br>2- HSF Matrices- hnRNP A1<br>3- IIEs from Zhang et al. |  | Creation of an exonic ESS site.<br>Potential alteration of splicing. |
| ESE Site Broken | 1-ESE-Finder -SRp55<br>2- EIEs from Zhang et al.<br>3-HSF Matrices - 9G8<br>4- ESE-Finder - SC35 |  | Alteration of an exonic ESE site.<br>Potential alteration of splicing. |
| **B) *LMTK2*-rs7791463 (G>A)**** | | | |
| New Acceptor Site | 1- HSF Matrices |  | Activation of an exonic cryptic acceptor site, with the presence of one or more cryptic branch point(s).<br>Potential alteration of splicing. |
| New ESS Site | 1- Sironi et al. - Motif 1<br>2- Sironi et al. -Motif 2<br>3-HSF Matrices -hnRNP A1 |  | Creation of an exonic ESS site.<br>Potential alteration of splicing. |

* HSF results showed that rs4794758 (A>G) transition led to a crate of one ESE site in the *HNF1B*-hnRNA.
** Transition of rs7791463 (G>A) led to the creation of one ESE site, one acceptor site, and up to multi branch points in the *LMTK2*-hnRNA.

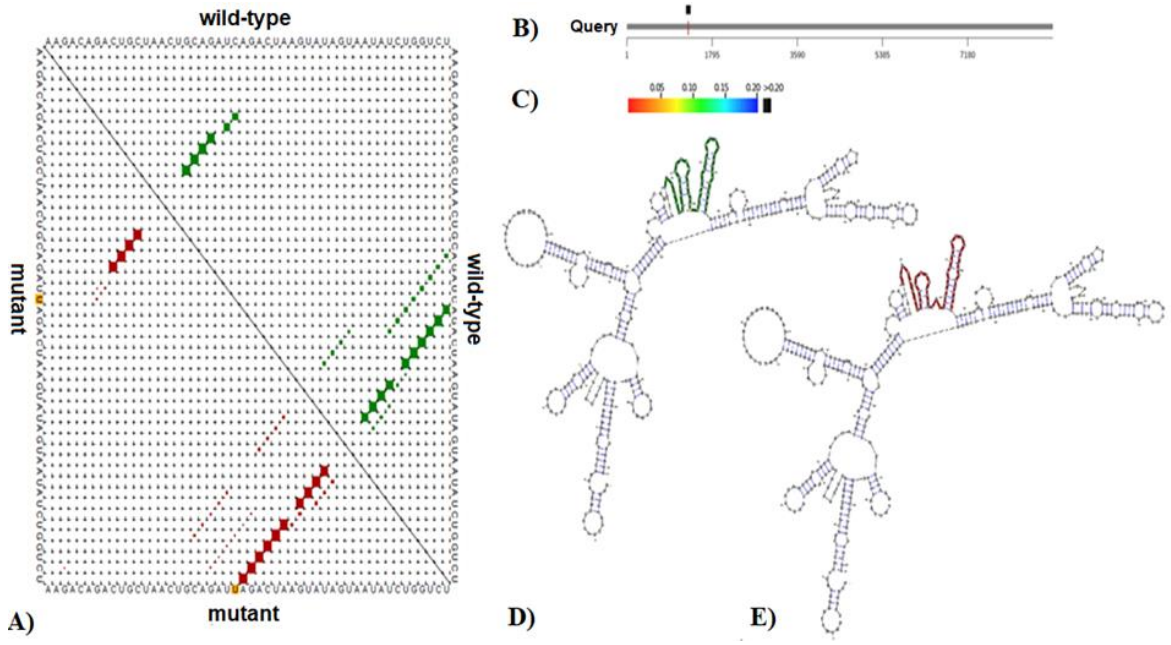



**Figure 2.** Prediction of RNAsnp server about the effect of rs7791463 transition on LMTK2-mRNA: A) Peripheral region (1318-1269) with the greatest difference between the wild-type mRNA and the molecule containing the modified nucleotide. The upper and lower triangles show the match pair probabilities in the wild and mutant sequences, respectively. The substituted nucleotide is shown in yellow color; B) Graphical summary of this analysis. The region affected by SNP is shown in black due to having a probability level higher than 0.2, which means that there was no significant change in the mRNA structure; C) P-value colored guide. D) The second optimal structure of the wild-type sequence in the peripheral region of the SNP (1092-1492) with a minimum free energy of -108.40 kcal/mol is shown in green; E) The second optimized structure of the sequence containing the change in the peripheral region of the SNP (1092-1492) with a minimum free energy of -108.10 kcal/mol is shown in red.

BPH affects 8% of older men in their fourth decade and up to 90% in their ninth decade (Langan, 2019). Asian men have 1 in 13 prostate cancer risks, and 1 in 44 dies (Lloyd et al., 2015).

In the 2020 years, prostate cancer was the second most common cancer diagnosed worldwide and the fifth leading cause of cancer-related deaths among men (Wang et al., 2022). It is Iran's third most common visceral cancer and the seventh leading cause of cancer death (Jemal et al., 2011). Prostate cancer risk factors include age, race, and positive family history (Leitzmann et al., 2012). Evidence shows that *IGFBP-5*, *DAN1*, *RAB5A*, *HNF1B*, and *LMTK2* play essential roles in prostate cancer (Dhanasekaran et al., 2001; Harries et al., 2010).

This study examined the relationship between rs4794758 and rs7791463 polymorphisms and prostate cancer and benign hyperplasia in Iranian men. Additionally, a bioinformatics analysis was conducted. Based on our study, rs4794758 polymorphisms increase prostate cancer risk, while rs7791463 polymorphisms do not. No polymorphisms were associated with BPH risk.

## Discussion

Genome-wide association studies reveal numerous SNPs in non-protein-coding regions, impacting epigenetics, histone modifications, and mRNA expression (Naghiyan Fesharaki and Sisakhtnezhad, 2022; Colagar et al., 2023). In cancer samples, the rs4430796 single nucleotide polymorphism in *HNF1B* intron 2 is more prevalent, with carriers having higher Gleason scores and pathological stages (Helfand et al., 2014). Men with two risk alleles are four times more likely to develop early prostate cancer (Levin et al., 2008). The study found that the rs4794758 polymorphism was more strongly associated with prostate cancer risk than the previously reported rs11649743 polymorphism (Berndt et al., 2011; Ross-Adams et al., 2016). Reports show polymorphisms in intron 9 of *LMTK2* are associated with prostate cancer risk (Eeles et al., 2008). Environmental, geographical, and racial factors can affect results.

Overexpression of *HNF1B* in cancer cells may suppress tumor growth (Solovieff et al., 2013), but this protective effect is lost during prostate cancer progression due to promoter methylation (Dan et al., 2019). The *HNF1B* gene regulates *ECI2* expression, affecting *NR4A1* and *HSPD1* expression (Hamid et al., 2008). The LMTK2 protein, involved in normal and pathological conditions (Inoue et al., 2008; Eeles et al., 2008; Manser et al., 2012), has a 68% decrease in expression in cancer tissues compared to benign tissues (Harries et al., 2010). LMTK2 regulates androgen receptors (Puri et al., 2010), PSA, VEGF production (Shah et al., 2015), and TGF synthesis (Manser et al., 2012), contributing to cancer susceptibility and progression (Conti et al., 2016).

According to computer analysis, the *HNF1B*-rs4794758 polymorphism does not significantly alter the structure of mRNA. However, the splicing pattern for hnRNA may be changed by establishing an ESS site and an acceptor site and breaking an existing ESE site. Additionally, it changes the reading frame and creates a binding site for transcription factor TBP. According to a comprehensive bioinformatic analysis of the *LMTK2*-rs7791463 polymorphism, there is a significant alteration in the structure of mRNA. It affects hnRNA splicing by establishing multiple ESS sites, acceptor sites, and branch point motifs. It may be possible to understand the molecular effects of genetic polymorphism through bioinformatics studies.

## Conclusion

According to this study, the HNF1B- rs4794758 polymorphism is associated with prostate cancer but not benign prostatic hyperplasia. Bioinformatics research reveals that this polymorphism may alter hnRNA splicing patterns and the reading frame, affecting transcription factor binding sites. While it may be a suitable biomarker for prostate cancer in men, it is not ideal for BPH. Furthermore, this study indicated that the LMTK2-rs7791463 polymorphism is not associated with prostate cancer or benign prostatic hyperplasia, as it significantly affects mRNA structure and hnRNA splicing patterns. Thus, it is unsuitable for a biomarker to determine whether or not a man is at risk of prostate cancer or BPH in men. To obtain more accurate results, further studies on a broader population will be necessary, incorporating more risk factors and experimentally validating for in silico analysis.

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**Conflict of Interests**

The authors declare that there is no conflict of interest.

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## Supplementary Figures

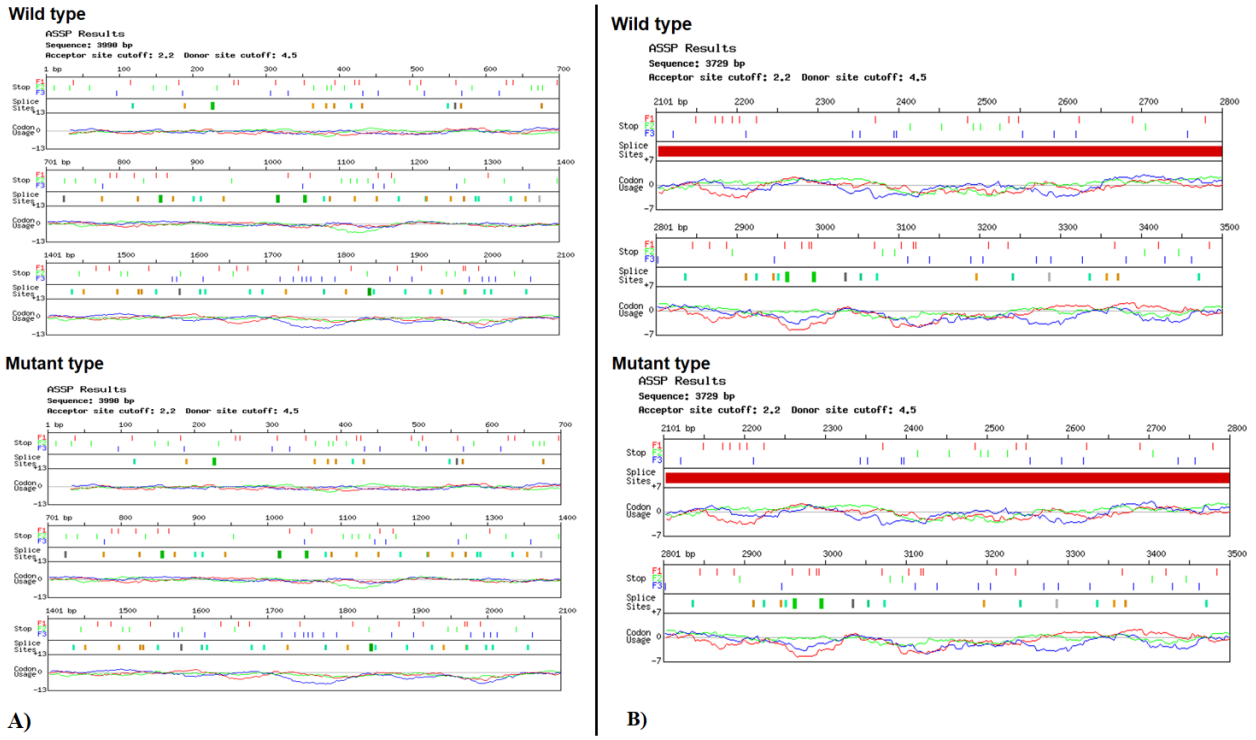


**Figure S1.** Prediction of rs4794758 (A>G) and rs7791463 (G>A) transition effects on the *HNF1B*-mRNA and *LMTK2*-mRNA by ASSP tool: A) showed that transition of rs4794758 (A>G) is not effects on the mRNA processing; B) Transition of rs7791463 (G>A) on the *LMTK2*-mRNA led to three open reading fram.

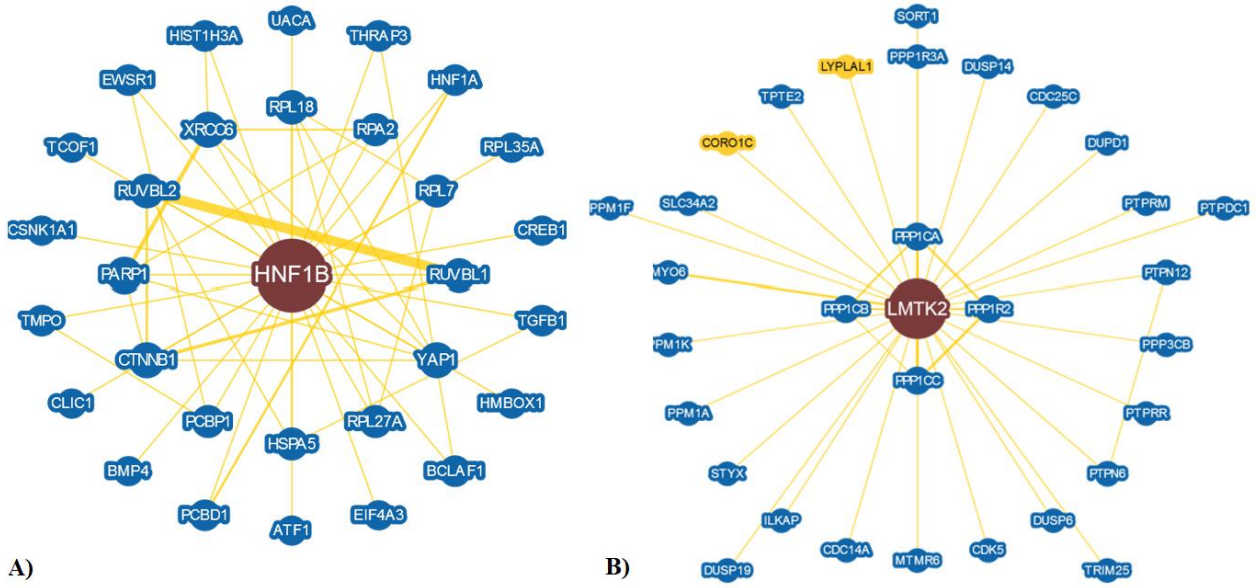


**Figure S2.** Human HNF1B and LMTK2 interactions with other proteins and the network obtained from BioGRID: A) Intraction of HNF1B with other proteins; Intraction of LMTK2 with other proteins; **ATF1**: Activating Transcription Factor 1; **BCLAF1**: BCL2 Associated Transcription Factor 1; **BMP4**: Bone Morphogenetic Protein 4; **CLIC1**: Chloride Intracellular Channel 1; **CREB1**: CAMP Responsive Element Binding Protein 1; **CSNK1A1**: Casein Kinase 1 Alpha 1; **CTNNB1**: Catenin Beta 1; **EIF4A3**: Eukaryotic Translation Initiation Factor 4A3; **EWSR1**: EWS RNA Binding Protein 1; **HIST1H3A**: Histone Cluster 1 H3 Family Member A; **HMBOX1**: Homeobox Containing 1; **HNF1A**: HNF1 Homeobox A; **HSPA5**: Heat Shock Protein Family A (Hsp70) Member 5; **PARP1**: Poly(ADP-Ribose) Polymerase 1; **PCBD1**: Pterin-4 Alpha-Carbinolamine Dehydratase 1; **PCBP1**: Poly(RC) Binding Protein 1; **RPA2**: Replication Protein A2; **RPL7**: Ribosomal Protein L7; **RPL18**: Ribosomal Protein L18; **RPL27A**: Ribosomal Protein L27a; **RPL35A**: Ribosomal Protein L35a; **RUVBL1**: RuvB Like AAA ATPase 1; **RUVBL2**: RuvB Like AAA ATPase 2; **TCOF1**: Treacle Ribosome Biogenesis Factor 1; **TGFB1**: Transforming Growth Factor Beta 1; **THRAP3**: Thyroid Hormone Receptor Associated Protein 3; **TMPO**: Thymopoietin; **UACA**: Uveal Autoantigen With Coiled-Coil Domains And Ankyrin Repeats; **XRCC6**: X-Ray Repair Cross Complementing 6; **YAP1**: Yes Associated Protein 1.; **CDC14A**: Cell Division Cycle 14A; **CDC25C**: Cell Division Cycle 25C; **CDK5**: Cyclin Dependent Kinase 5; **DUPD1**: Dual Specificity Phosphatase And Pro Isomerase Domain Containing 1; **DUSP6**: Dual Specificity Phosphatase 6; **DUSP14**: Dual Specificity Phosphatase 14; **DUSP19**: Dual Specificity Phosphatase 19; **ESR2**: Estrogen Receptor 2; **ILKAP**: ILK Associated Serine/Threonine Phosphatase; **MTMR6**: Myotubularin Related Protein 6; **MYO6**: Myosin VI; **PPM1A**: Protein Phosphatase, $Mg^{2+}/Mn^{2+}$ Dependent 1A; **PPM1F**: Protein Phosphatase, Mg2+/Mn2+ Dependent 1F; **PPM1K**: Protein Phosphatase, $Mg^{2+}/Mn^{2+}$ Dependent 1K; **PPP1CA**: Protein Phosphatase 1 Catalytic Subunit Alpha; **PPP1CB**: Protein Phosphatase 1 Catalytic Subunit Beta; **PPP1CC**: Protein Phosphatase 1 Catalytic Subunit Gamma; **PPP1R2**: Protein Phosphatase 1 Regulatory Inhibitor Subunit 2; **PPP1R3A**: Protein Phosphatase 1 Regulatory Subunit 3A; **PPP3CB**: Protein Phosphatase 3 Catalytic Subunit Beta; **PTPDC1**: Protein Tyrosine Phosphatase Domain Containing 1; **PTPN6**: Protein Tyrosine Phosphatase, Non-Receptor Type 6; **PTPN12**: Protein Tyrosine Phosphatase, Non-Receptor Type 12; **PTPRM**: Protein Tyrosine Phosphatase, Receptor Type M; **PTPRR**: Protein Tyrosine Phosphatase, Receptor Type R; **SLC34A2**: Solute Carrier Family 34 Member 2; **SORT1**: Sortilin 1; **STYX**: Serine/Threonine/Tyrosine Interacting Protein; **TPTE2**: Transmembrane Phosphoinositide 3-Phosphatase And Tensin Homolog 2; **TRIM25**: Tripartite Motif Containing 25; **LYPLAL1**: Lysophospholipase Like 1 (Mus musculus); **CORO1C**: Coronin 1C (Mus musculus).