

Research Article

The Association Between Leptin (-2548 G>A, rs7799039)/Leptin Receptor (223 A>G, rs1137101) Polymorphisms and Prostate Cancer

Ahmad Mohammad Khalil^{1,*} , Alaa Helo Abu Helo² ,
Lulu Husni Alsheikh Hussein¹ , Mohammad Aeman Kazali¹ 

¹Department of Biological Sciences, Yarmouk University, Irbid, Jordan

²Department of Biotechnology and Genetic Engineering, Philadelphia University, Amman, Jordan

Abstract

Background: The pathophysiology of Prostate Cancer (PCa) involves a complex interplay of environmental and genetic factors. PCa diagnosis faces challenges of false-positive outcomes, emphasizing the necessity for revising the available screening methods for PCa. Single-nucleotide polymorphisms (SNPs) have been linked with PCa and constitute a key risk factor in prostate carcinogenesis. **Objective:** This research aimed at examining the association between susceptibility to PCa and polymorphisms of the *Leptin* (*LEP*) gene -2548 G>A (rs7799039) and *LEP receptor* (*LEPR*) gene 223 A>G (rs1137101). **Methods:** A total of 66 patients and 34 controls of a Jordanian population were recruited. DNA was isolated from blood leucocytes. Polymorphism analyses were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approaches. **Results:** The mutant allele (A) and genotype (AA) frequencies of the *LEP* SNP (-2548 G>A, rs7799039) were significantly higher in the PCa patients (59.1% and 37.9%, respectively) compared to those in the control group (45.6% and 20.6%, respectively) ($P = 0.039$; OR = 1.88, 95%CI = 1.02 - 2.89 and $P = 0.043$; OR = 2.86, 95%CI = 1.77 - 5.92, respectively). This indicates that this SNP was associated with an overall increased risk of PCa. In contrast, the *LEPR* 223 A>G was not. Further stratification of the PCa group showed that the incidence of both SNPs was correlated with high serum PSA levels and metastasis risk. The results with age and Gleason biopsy score were not conclusive because the trend observed did not reach significance. **Conclusions:** The findings support our hypothesis regarding the association of the polymorphism in the *LEP* gene in prostate carcinogenesis (the A and AA frequencies, compared with the control, were associated with a 1.3-fold and a 1.8-fold increased risk for PCa, respectively). However, there was no association between the *LEPR* (rs1137101) gene variant and PCa risk.

Keywords

Leptin, Leptin Receptor, Prostate Cancer, Leptin SNP (-2548 G>A); Leptin Receptor SNP (223 A>G)

1. Introduction

Prostate cancer (PCa) is the second most common type of malignancy (after skin cancer) in men worldwide [1]. It is

the second leading cause of cancer death in males (after lung cancer) [2]. A recent report has projected that the number of

*Corresponding author: ahmad_k@yu.edu.jo (Ahmad Mohammad Khalil)

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PCa cases will rise from 1.4 million in 2020 to 2.9 million by 2040 [3]. The financial cost of PCa treatment is growing fast; for example, in the U.S., it will be beyond \$245 billion by 2030. In Jordan, based on the WHO data published in 2017, PCa deaths reached 0.72% of total deaths [4].

The exact pathophysiology of PCa remains not fully well-known but appears to involve a complex interplay of factors, including age, lifestyle/environmental factors, inflammation, as well as familial history, and genetic background [5]. Despite promising discoveries, no drugs directly targeting these mechanisms have been approved yet. PCa diagnosis persists in facing challenges of false-positive results, over-detection, and overtreatment [2], emphasizing the need to revise the available screening methods for PCa.

Genetic variants that add to disease development risk are continuously being acknowledged [6]. Single-nucleotide polymorphisms (SNPs) are the most frequent genetic alterations. They represent different forms of individual change of nucleotides in the genome (gene promoters, exons, introns, as well as 5'-and 3'-untranslated regions (UTRs)) [7]. About 100 well-documented SNPs have been linked with PCa and constitute a key risk factor in prostate carcinogenesis [2, 8]. Yet unraveling their functional implications remains elusive.

The SNP rs7799039 (-2548 G>A) in the 5'-promoter region of the Leptin (LEP) gene results when a G replaces an A at nucleotide 2548 upstream of the ATG start site [9]. This SNP is one of the most frequently related to genotype differences in the development of PCa [10]. It has been reported that the LEP (-2548) A allele enhances gene expression, consequently increasing the level of LEP protein in the serum [10].

The SNP (rs1137101) (223 A>G) is one of the most common missense SNPs in the LEP receptor (LEPR) gene. It involves a G transition instead of A at nucleotide 668 from the start of codon 223 in exon 6 [11]. The introduction of positive charges (arginine) changes the ability of LEPR to fold properly and consequently impairs LEP-binding activity, leading to serious changes in LEP-LEPR signaling pathways that activate the proliferation, angiogenesis, metastasis, and chemoresistance as well as inhibiting epithelial cell apoptosis [12]. Expression levels of LEP/ LEPR as potential biomarkers for risk of PCa development and aggressiveness have been assessed [12]. The expression of both genes was

correlated significantly with prostate malignancy [13]. Consequently, researchers suggested that LEP/LEPR expression could be a potential prognostic biomarker for prostate malignancy [13]. One study [9] reported in a meta-analysis [14] found that the LEP-2548 G>A genotype was significantly associated with an overall increased risk and aggressiveness of PCa. In contrast, the LEPR 223 A>G was not.

In view of insufficiency and inconsistency regarding the link between common genetic polymorphisms and PCa trajectories, this study was carried out to assess the associations between polymorphisms of LEP (-2548 G>A) and LEPR (223 A>G) genes and PCa development in a sample of the Jordanian population. The outcome might help in developing better risk-assessment approaches, as well as creating novel targeted therapies. Applying prediction and diagnostic importance of the disease and its associated risks could lower the number of sufferers and reduce the mortality rates.

2. Patients and Methods

2.1. Study Population

A total of 100 men were recruited for this study. The first group consisted of 66 (mean age = 69 ± 10.2 years) PCa patients, while the second group included 34 normal participants of younger age (40 ± 12.6 years). Individuals in the control group were randomly selected from males attending the Urology outpatient clinic. Prostate cancer patients were diagnosed by an oncologist and staged by a histopathologist in the Urology Department at King Abdullah University Hospital (Irbid, Jordan). The exclusion criteria of the control group were previous malignancy or chronic diseases, or abnormal laboratory data. Patients with benign prostatic hyperplasia were excluded as a comparison group. Clinical data were collected from the patient's medical records, and demographic information was collected through a well-prepared, comprehensive, structured in-person questionnaire. An informed consent form was signed by all participants before the study. Table 1 shows the age distribution and the clinical characteristics of the PCa subjects involved in this study.

Table 1. Basic clinical characteristics of the prostate cancer cases in the Jordanian population enrolled in this study.

Characteristic	Cases N=66(100%)
Mean age * (years)	
45-58	6(9.1)
59-73	29(43.9)
74-88	31(47.0)
Total	66 (100%)

Characteristic	Cases N=66(100%)
Prostate Specific Antigen (ng/μl)	
0.0-4.0	19(28.8)
4.1-10	26(39.4)
>10.0	21(31.8)
Total	66(100%)
Gleason score * (N=%)	
Low grade (5-6)	20(30.3)
Intermediate grade (7-8)	29(43.9)
High grade (9-10)	17(25.8)
Total	66(100%)
Tumor stage N (%)	
Localized	52(78.8)
Metastatic	14(21.2)
Total	66(100%)

* Younger groups were excluded since prostate cancer is rare in men younger than 40 years of age [14].

** The Gleason scoring system is one of the widely used methods to determine the prognosis of PCa. It is based on histopathology and correlates with prostate-specific antigen (PSA) value, clinical and pathological staging, and incidence of lymph nodes and metastases. Scores 1 and 2 are now not often used, and 3 is the lowest grade in clinical usage (the system has intentionally not been updated to allow back comparison to historical series) [3].

2.2. SNP Selection

The two SNPs *LEP* (-2548 G>A) and *LEPR* (223 A>G) investigated in this study were selected from a public database (<https://ist.medisapiens.com/>, accessed in January 2021) as well as through two international databases (International HapMap database (version: release #27; <http://www.hapmap.org>) and NCBI dbSNPs in the *LEP* and *LEPR* gene regions. Also, the two SNPs were chosen based on the best evidence from published sources on the genetics of PCa, focusing on candidate gene association research [9, 15].

2.3. Blood Collection

From each individual, 10 ml of venous peripheral blood was obtained in two Vacutainer tubes: a plain tube for measurement of serum PSA and another EDTA-coated tube for detection of *LEP* and *LEPR* SNPs. Serum PSA level was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Sigma-Aldrich, St. Louis, MO, USA). The second blood sample was stored at -80 °C until the analyses.

2.4. DNA Extraction

Total genomic DNA was extracted from leucocytes using

the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's procedure. Briefly, 500 μl of whole blood was lysed in 1500 μl of Cell Lysis Solution at room temperature (RT) for 10 min. After centrifugation for one min at 14,000 g, the white blood cell pellet was lysed using 500 μL of Nuclei Lysis Solution. The solution was incubated at 37 °C until the clumps were disrupted. Then, 2.5 μl of RNase Solution and 166 μl of Protein Precipitation Solution were added, followed by vigorous vortexing for a few sec. The protein was precipitated by centrifugation at 13,000 g for 5 min. The supernatant was then transferred into a sterile Eppendorf tube containing 670 μl of isopropanol, and the tube was inverted a few times until white thread-like strands of DNA formed a visible mass. The DNA was precipitated by centrifugation at 13,000 g for 4 min, and the supernatant was discarded. The DNA pellet was washed with 70% ethanol. Following centrifugation at 13,000 g for 4 min, the pelleted DNA was air-dried. Then, 50 μl of DNA hydration solution was added, and the sample was incubated at 65 °C for 30 min to dissolve the DNA.

2.5. DNA Amplification

The method of restriction fragment length polymorphism (RFLP) was carried out after the polymerase chain reaction (PCR) was used. The amplification procedure was performed with a 30 μl reaction mixture containing 15 μl master mix

(2X) (Promega, USA), 7.5 µl nuclease-free water (NFW), 3 µl F primer, 3 µl R primer, and 1.5 µl of genomic DNA. Amplification was performed using a thermal cycler (Swift™ MaxPro, Singapore). To verify the amplification of

the wanted fragments, all samples were electrophoresed on a 1.5% agarose gel after PCR. The amplification conditions, characteristics, and sequences of primers are presented in [Table 2](#) according to previous studies [16, 17].

Table 2. Amplification protocol for the selected adipokine genes: LEP -2548 G>A and LEPR 223 A>G.

Gene poly-morphism	dbSNP ID	Cytogenetic location	Gene region	SNP location (Base Change)	Primer sequence (5'-3')	Amplification conditions
LEP	rs7799039	7q31.3	Promotor	-2548 G>A	F: TTTCCTGTAATTTTCCC GTGAG R: AAAGCAAAGACAGGCA TAAAAA F: ACCCTTTAA- GCTGGGTGTCCCAA- TAG R: AGCTAGCAAA- TATTTTGTAAGCAATT	Initial incubation at 95 °C (5 min) 36 amplification cycles of incubation at 95 °C (30 sec) Annealing at 59 °C (30 sec) Extension at 72 °C (30 sec) Final extension at 72 °C (5 min)
LEPR	rs1137101	1p31	Exon 6	223 A>G (223 Q>R)		

Abbreviations: LEP: Leptin; LEPR: LEP receptor.

2.6. DNA Genotyping

The PCR products of the SNPs LEP (242 bp) and LEPR (416 bp) were detected on 3% agarose gels in TAE buffer containing ethidium bromide. The gels were run for 50 min (Consort EV243 Electrophoresis Power Supply, UK). The amplified products of the LEP SNP (242 bp) were digested with the restriction enzyme Hha1 (Cfo1) (Thermo Scientific, USA). The reaction mixture consisted of a 12 µl aliquot of PCR product (DNA), 5 µl NFW, 2 µl reaction buffer, and 1 µl (10 U/µl) of restriction enzyme ([Table 3](#)). The restricted fragments were electrophoresed and analyzed on 3% agarose gel (90 V) for 60 min. Bands in gels were stained with ethidium bromide for visualization under ultraviolet light. Substitutions of G with A at 2548 in the promoter of the LEP gene establish a recognition site for Hha1. Digestion by Hha1 showed one band of 242 bp in the G allele in the normal (wild type). Three distinct bands of 242 bp, 182 bp, and 60 bp in the heterozygous individual (GA), and two separate

bands (182 and 60 bp) in the A allele in the mutant homozygote genotype (GG) ([Figure 1](#) and [Table 3](#)).

Likewise, the amplified products of the LEPR gene (223 A>G) SNP (416 bp) were digested overnight with the MspI restriction enzyme (Thermo Scientific, USA). The reaction mixture contained 12 µl aliquot of PCR product (DNA), 5 µl NFW, 2 µl reaction buffer, and 1 µl (10 U/µl) of the restriction enzyme ([Table 3](#)). The restricted fragments were electrophoresed and analyzed on a 2.5% agarose gel (90 V) for 60 min. Bands in gels were stained with ethidium bromide for visualization under ultraviolet light. Substitution of A with G at nucleotide 668 in exon 6 of the LEPR gene establishes a recognition site for MspI. Digestion by MspI showed one band of 416 bp in the A allele in the normal (wild type) homozygous genotype (AA). Three distinct bands of 416 bp, 291 bp, and 125 bp in the heterozygous individual (AG), and two separate bands (291 bp and 125 bp) in the G allele in the mutant homozygote genotype (GG) ([Figure 2](#) and [Table 3](#)).

Table 3. PCR amplification fragments, the incubation and deactivation conditions, and the size of the digestion products of the LEP SNP 2548 G>A and the LEPR SNP 223 A>G.

Genetic polymorphism	Amplicon size (bp)	Restriction enzyme	Reaction conditions	Size of the digestion fragments (bp)
LEP (-2548)	242	Hha1	Incubation: 16 h at 37 °C	GG: 242 bp; GA: 242 bp, 182 bp, and 60 bp;

Genetic polymorphism	Amplicon size (bp)	Restriction enzyme	Reaction conditions	Size of the digestion fragments (bp)
G>A				AA: 182 bp and 60 bp
LEPR (223) A>G (223Q>R)	416	<i>MspI</i>	Incubation: 16 h at 37 °C	AA: 416 bp; AG: 416 bp, 291, and 125 bp; GG: 291 bp and 125 bp

Abbreviations: LEP: Leptin; LEPR: LEP receptor.

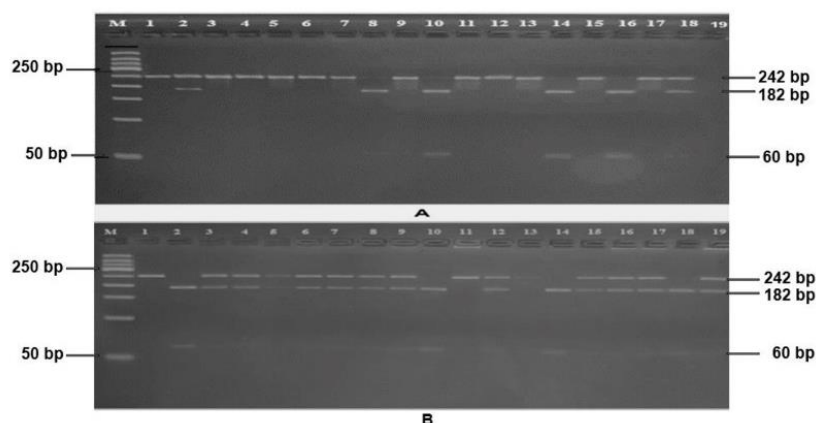


Figure 1. Representative gels were used for genotype analysis of the LEP SNP (rs7799039; - 2548 G>A). Digested products were run on 3% agarose gel and visualized under UV light. Lane M: 50 bp DNA marker ladder (QIAGEN, Germany). (A) Healthy control samples. Lanes 1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 17 (Uncut fragments 242 bp) homozygous (AA) mutant. Lanes 2 and 18 (242, 182, 60 bp): heterozygous (GA). Lanes: 8, 10, 14, 16 (182, 60 bp): homozygous (GG) wild type; Lane 19: NFW blank (negative control). (B) Prostate cancer cases. Lanes 1, 11, and 13 (the uncut fragment 242 bp) homozygous (AA) mutant. Lanes 2, 10, 14, and 18 (182 and 60 bp): homozygous (GG) wild type; lanes 3, 4, 5, 6, 7, 8, 9, 12, 15, 16, 17, 19 (242, 182, and 60 bp): heterozygous (GA).

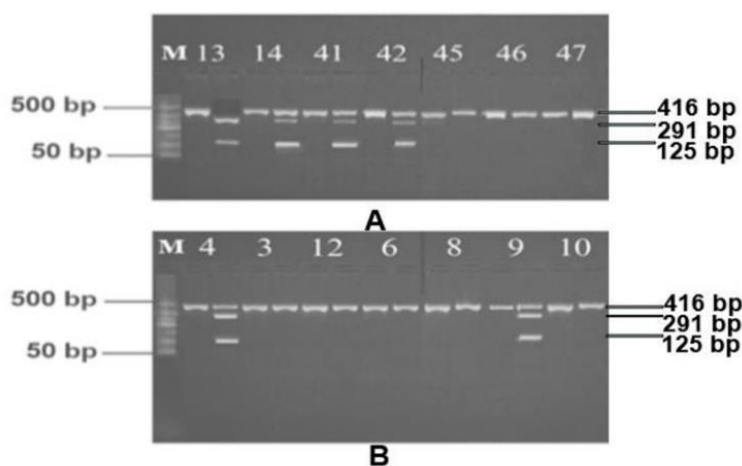


Figure 2. Illustrative gels of the digestion fragments and genotypes for LEPR SNP (rs1137101; 223 A>G or 223 Q>R). Digested products were run on a 2.5% agarose gel and visualized under UV light. Lane M: 50 bp DNA ladder (QIAGEN, Germany). Left lanes: Undigested PCR fragments. Right lanes: Digested amplicons. (A) Healthy control samples. Lane 13: (291, 125 bp) homozygous (AA) mutant; lanes: 14, 41, 42 bp (416, 291, 125 bp) heterozygous (AG); Lanes: 45, 46, 47 (the uncut fragment 416 bp) homozygous (GG) mutant. (B) Prostate cancer cases. Lanes: 4, 9 (416, 291, 125 bp): heterozygous (AG); Lanes: 3, 12, 6, 8, 10 (the uncut fragment 416 bp) homozygous (GG) mutant.

To evaluate the reliability of genotyping, twice sampling of RFLP-PCR in approximately 10% of randomly selected samples was performed.

2.7. Statistical Analysis

The statistical program for social sciences (SPSS) for Windows v. 21.0 was used for data analysis. The sample characteristics were described by means $\pm$ SD and frequencies (%). The distribution of SNP alleles and genotypes was assessed separately for PCa cases and non-cancerous controls. The deviation of genotype frequencies from the Hardy-Weinberg equilibrium (HWE) among controls was assessed by the Chi-square test (χ^2). The two SNPs were in HWE. The Chi-square and Fisher's exact tests were used to assess the association between the allelic and genotypic frequencies with clinical variables, including age, Gleason score, metastasis, and PSA level, where continuous clinical variables were converted into clinically relevant categories. The strength of associations of LEP-2548 G>A and LEPR 223 A>G SNPs with PCa risk was examined by odds ratios (ORs) with their corresponding 95% confidence intervals (CIs). The ORs and the CIs were calculated by binary logistic regression. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Genotype and Allele Distributions

The frequencies of genotypes and alleles of both LEP and LEPR polymorphisms in the surveyed individuals followed the HWE. The HWE test for the two SNPs revealed no significant deviation ($p=1.00$ in the PCa subjects and $p=0.76$ in the controls).

3.2. LEP SNP (rs7799039; -2548 G>A)

The distribution of the genotype and allele frequencies of the LEP (-2548 G>A) polymorphism and control groups is presented in Table 4. Compared with the non-cancer group for this SNP, the AA genotype and A allele were more common in patients with PCa. (37.9% versus 20.6% and 59.1% versus 45.6%, respectively) (OR=2.86; CI= 1.77-5.92; $P=0.043$ and OR=1.88; CI= 1.02-2.89; $P=0.039$). Furthermore, a significant difference was observed in the LEP GA genotype between subjects with and without PCa (42.4% and 50% respectively) (OR = 1.74; $p = 0.051$)

Table 4. Association between genotypes and alleles of the LEP and the LEPR gene variants and the risk of prostate cancer.

Gene polymorphism	Genotypes and alleles	PCa cases N= 66 (100%)	Controls N= 34 (100%)	OR (95% CI) P Value
LEP (-2548 G>A)	GG	13(19.7)	10(29.4)	1.0 (reference)
	GA	28(42.4)	17(50.0)	1.74 (0.96-3.20) 0.051
	AA	25(37.9)	7(20.6)	2.86 (1.77-5.92) 0.043
	G	54(40.9)	35(54.4)	1.0 (reference)
	A	78(59.1)	29(45.6)	1.88 (1.02-2.89) 0.039
	AA	32 (48.5)	16(47.1)	1.0 (reference)
LEPR 223A>G (223 Q>R)	AG	26(39.4)	13(38.2)	0.76 (0.31-1.86) 0.837
	GG	8(12.1)	5(14.7)	1.17 (0.29-5.92) 0.544
	A	90(68.2)	45(66.2)	1.0 (reference)
	G	42(31.8)	23(33.8)	0.96 (0.51-1.83) 0.889

Data are presented as the number of cases with frequency in parentheses. Abbreviations: CI: Confidence interval; LEP: Leptin; LEPR: LEP receptor; OR: Odds ratio; PCa: Prostate cancer.

3.3. LEPR SNP (rs1137101; 223 A>G)

The distribution of the genotype and allele frequencies appears in Table 4. Compared with the noncancerous control, there were no differences in the frequencies between mutant allele G and mutant homozygous (GG) genotype in the

LEPR 223 G>A (rs1137101) SNP (31.8% and 12.1% versus 33.8% and 14.7%, respectively) (OR = 0.96; CI=0.51-1.83; P = 0.889 and OR= 1.17; 0.29-5.92; P=0.544). Similarly, no significant difference was observed in the LEPR AG genotype between subjects with and without PCa (39.4% and 38.2%, respectively) (OR = 0.76; p = 0.837).

Table 5. The relationship between genotype *LEP* -2548 G>A and *LEPR* 223 A>G polymorphism and pathological features of prostate cancer.

Parameter	LEP -2548 G>A			P Value*	Parameter	LEPR 223 A>G			P Value*
Age (years) N=64(100%)	45-58	59-73	74-88	0.443	Age (years) N=64(100%)	45-58	59-73	74-88	0.358
Genotype	6(9.3)	28(43.8)	30(46.9)		Genotype	6(9.4)	27(42.2)	31(48.4)	
GG	2(33.3)	5(17.9)	4(13.3)		AA	4(66.6)	8(29.6)	12(38.7)	
GA	3(50.0)	9(32.1)	14(46.7)		AG	1(16.7)	16(59.3)	14(45.2)	
AA	1(1.7)	14(50.0)	12(40.0)		GG	1(16.7)	3(11.1)	5(16.1)	
PSA (ng/μl) N=66(100%)	0.0-4.0	4.1-10	>10	0.818	PSA (ng/μl) N=66(100%)	0.0-4.0	4.1-10	>10	0.847
Genotype	19(28.8)	26(39.4)	21(31.8)		Genotype	19(24.2)	26(45.4)	21(30.4)	
GG	11(57.9)	6(23.1)	5(23.8)		AA	6(31.6)	12(46.2)	9(42.8)	
GA	5(26.3)	9(34.6)	6(28.6)		AG	7(36.8)	9(34.6)	8(38.1)	
AA	3(15.8)	11(42.3)	10(47.6)		GG	6(31.6)	5(19.2)	4(19.1)	
Gleason score N=58(100%)	Low grade (5-6)	Intermediate grade (7-8)	High grade (9-10)	0.501	Gleason score N=58(100%)	Low Grade (5-6)	Inter-mediate grade (7-8)	High grade (9-10)	0.381
Genotype	15	27(46.5)	16(27.6)		Genotype	16(27.6)	22(37.9)	20(34.5)	
GG	(25.9)	3(11.1)	5(31.3)		AA	7(43.8)	22(37.9)	9(45.0)	
GA	4(26.7)	13(48.2)	5(31.3)		AG	3(18.8)	9(40.9)	7(35.0)	
AA	5(33.3)	11(40.7)	6(37.4)		GG	6(37.4)	10(45.5)	4(20.0)	
Metastasis N=65(100%)	Yes	No		0.925	Metastasis N=65(100%)	Yes	No		0.147
Genotype	13	52 (80.0)			Genotype	13(20.0)	52(80.0)		
GG	(20.0)	11(16.9)			AA	4(30.8)	27 (51.9)		
GA	2(15.4)	21(32.3)			AG	6(46.1)	21(40.4)		
AA	5(38.5)	20(30.8)			GG	3(23.1)	4(7.7)		

Data are presented as a number of cases with frequency in parentheses. CI, confidence interval; LEP: Leptin; LEPR: Leptin receptor; OR: Odds ratio. * P-value from Chi-square test or Fisher's exact test as appropriate

3.4. Association Between SNP Genotypes and Clinical Characteristics

Table 5 shows the *LEP*-2548 G > A as well as *LEPR* 223 A>G data stratified based on clinical and biochemical parameters in the PCa patients. In both SNPs, neither the age nor the Gleason score showed a clear relationship with the incidence of PCa.

The results showed that relative to PCa patients with the mutant homozygous genotype of the studied *LEP* SNP, the incidence increased, but not significantly, with increasing serum PSA levels; 42.3%, and 47.6% for 4.1, and >10 ng/μl,

respectively (P=0.847) (Table 5). A reverse trend was observed in the case of *LEPR* 223 GG: 31.9%, 19.2%, 19.1%. In addition, for SNP *LEP* -2548 G>A, a lower rate of PCa metastasis was recorded in individuals carrying the mutant genotype as compared with those having the wild-type genotype (30.8% versus 46.1%). A reverse fashion was noted in the mutant genotype of *LEPR* 223 A>G SNP (23.1% metastatic and 7.7% local).

4. Discussion

Currently, there is an increasing trend towards identifying

predictive and presumably disease-associated SNPs. The present study examined, for the first time, the relationship between -2548 G/A polymorphism in the *LEP* gene and 223 A>G polymorphism in the *LEPR* gene with the risk for PCa among the Jordanian population. Our main finding is that the mutant allele (A) of the SNP *LEP* (rs7799039) -2548 G>A was more significantly predominant than in the PCa patient group. No significant difference was observed in the *LEPR* (A>G rs1137101, A223 A>G) genotype between subjects with and without PCa. This means that, among the Jordanian population under study, the *LEP* (rs7799039) is significantly associated with an increased risk of PCa, but the SNP *LEPR* has no clear effect on the PCa risk. To date, there is only a handful of studies that concentrate on the association of the two polymorphisms with susceptibility to PCa. In agreement with our data are those of three independent epidemiological research groups [9], which proposed that the *LEP* SNP at position -2548 was a predisposition locus for PCa. However, previously published data did not support this contention [9]. A more recent meta-analysis [18] concluded that *LEP* rs7799039 was related to an increased risk of PCa. In contrast, the *LEPR* rs1137101 variant was correlated with a reduced risk of PCa. Another study [19] showed a relationship between the *LEPR* A223G gene polymorphism with PCa. Thus, our results confirm and extend the published data [9, 15].

Another important result demonstrated in our study is the association between elevated serum PSA levels and carrying the *LEP* (-2548) mutant A allele. This result is consistent with previous studies regarding enhanced *LEP* gene expression, increased circulating leptin levels [16], and the development of PCa [8, 19]. The higher frequency among diseased men may suggest that the mutant allele may confer susceptibility to the development of PCa.

Another noteworthy finding in the current investigation is that the incidence of the mutant allele (A) of *LEP* (-2548), as well as that of *LEPR* 223G, was significantly greater in the metastatic group. This may indicate that the presence of the mutant allele possibly contributes to the aggressive metastasis of cancerous cells. In this regard, a few previous studies addressed the relationship between *LEP* and *LEPR* polymorphisms and PCa risk. In a previous study [20], the expressed levels of *LEPR* revealed a substantial association with localized PCa and metastatic PCa ($P < 0.05$). However, *LEPR* did not show a definite difference between benign prostatic hyperplasia (BPH) and PCa.

When our PCa patients were stratified according to Gleason scores, we couldn't reach conclusive results. Generally, higher Gleason scores were recorded in the literature among PCa-diseased men possessing the mutant allele, suggesting a positive interrelationship between elevated levels of *LEP* expression, highly differentiated malignant cells on one hand, and the incidence of occurrence of PCa [12]. Therefore, the relation of the two investigated polymorphisms with PCa risk may be unclear.

Another result found in the present investigation was the null relationship between age on one hand and the incidence

of *LEP* SNP (G>A, rs7799039) and *LEPR* gene (A>G, rs1137101) polymorphism. This result was unexpected because of the recent report revealing that the incidence of PCa increases with advancing age [21]. Prostate cancer is most frequently diagnosed among men aged 65 to 74 years, and the median age at diagnosis is 67 years [22]. This is correlated with age-specific increases in serum PSA levels and prostate size [21]. Prostate cancer is not often observed in men younger than 40 years. The frequency rises sharply with each decade thereafter. For instance, the chance of being diagnosed with PCa is 1 in 449 for males aged 49 years or younger, versus 1 in 10 for those aged between 65 and 84 years [23]. However, the rate for earlier age at the start in individuals having familial PCa exists; however, convincing proof for a difference in the clinical development or pathological features is lacking. Our research is the first attempt to analyze the effect of genetic polymorphisms in *LEP* and *LEPR* on the risk and aggressiveness of PCa. One strength of this research is the homogeneity of the investigated sample because the population is predominantly Arab (98%) [24]. This eliminates the influences on allele sequences that may result from different ethnic groups. SNPs such as *LEP* -2548 G>A and *LEPR* A>G have been shown to vary in multi-ethnic populations [9].

Several limitations in the current study should be addressed. First, the sample size (66 patients and 34 controls) is relatively small, which may limit the statistical power of the study. Therefore, there is a need for validation in a larger cohort. Truly normal samples could only be acquired at a much younger age relative to PCa cases (40 ± 12.6 years versus 69 ± 10.2 years), which could introduce bias. Second, the study is limited by time and geographical aspects: all participants were recruited from the same institution. The research team has been faced with some instrumental and administrative repetitive applications and reluctance in co-operation from several public and private clinics. Therefore, the researched population may not be representative since the question of selection bias cannot be excluded due to hospital-based controls. The next drawback is the small size of the included subjects, especially when the diseased group was stratified by age, PSA, or Gleason score. Further large sample-sized studies would provide more statistical power with a superior understanding of the association between PCa risk and *LEP* rs7799039 and *LEPR* rs1137101 polymorphisms. The scarcity of studies concentrated on the relationship between *LEP*-2548 G>A and *LEPR* A>G and PCa, so little information was available. The serum levels of *LEP* and *LPTR* were not assessed in this study, consequently precluding the ability to conduct genotype-phenotype correlation studies. In addition, the number of available epidemiological studies on the role of polymorphisms in the *LEP* and *LEPR* genes in PCa susceptibility in most populations was too small, and the details given in some reports were inadequate. There was considerable heterogeneity among study estimates, particularly cross-sectional evidence, implying that the true

effect estimates may vary between different populations. Consequently, it is recommended that the quantitative results be interpreted with caution and that sources of heterogeneity be thoroughly explored. More well-designed investigations with larger populations are warranted.

5. Conclusions

This work presents evidence-based data that support our hypotheses that the *LEP*-2548 G>A (rs7799039) polymorphism might be considered a risk genotype for the development of PCa cancer. Our results suggest that the minor (mutant) alleles of the *LEP*-2548 and *LEPR* 223 G are positively associated with metastatic-risk aggressiveness of PCa within the studied Jordanian population. The minor allele of the *LEP*-2548 G variant was significantly associated with increased PSA levels (ng/μl) in patients with PCa. The *LEP/LEPR* alone is unlikely to be a useful genetic biomarker for identifying risk or prognosis of this disease, but together with some available biomarkers may have stronger predictive value for PCa risk. Looking ahead, a demonstration of the clinical significance of the two studied SNPs has to be provided by results in PCa patient cohorts. However, it is important to continue investigating the applicability of SNPs in PCa progression and prediction to increase the molecular understanding of this cancer. A combination of the two SNPs with other genetic variants such as *LEP* SNPs (rs11761556, rs12706832, rs2071045, and rs2167270) and *LEPR* SNPs (rs10749754, rs1137100, rs1137101, rs13306519, rs8179183, rs1805096, rs3790434, rs3806318, and rs7518632) would also facilitate more precise prediction of PCa risk. In the current study, we have observed the PSA to have a protective role during PCa metastatic progression, though the biology underlying the higher metastatic potential for the Thr163 PSA still needs further investigation. Finally, although the method used for genotyping (PCR-RFLP) is appropriate; however, additional confirmatory techniques (e.g., sequencing) could strengthen the reliability of the results.

Abbreviations

A	Adenine
BP	Base Pair
BPH	Benign Prostatic Hyperplasia
CI	Confidence Interval
ELISA	Enzyme-linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic Acid
F	Forward
G	Guanine
HWE	Hardy-Weinberg Equilibrium
LEP	Leptin
LEPR	LEP Receptor
NFW	Nuclease-free Water
OR	Odds Ratio

PCR	Polymerase Chain Reaction
PCa	Prostate Cancer
PSA	Prostate-specific Antigen
R	Reverse
RFLP	Restriction Fragment Length Polymorphism
RT	Room Temperature
SNP	Single-nucleotide Polymorphism
SPSS	Statistical Program for Social Sciences
TAE	Tris-acetate-EDTA
UTRs	Untranslated Regions

Author Contributions

Ahmad Mohammad Khalil: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing

Alaa Helo Abu Helo: Data curation, Funding acquisition, Resources, Writing – original draft

Lulu Husni Alsheikh Hussein: Data curation, Investigation, Methodology, Software, Writing – original draft

Mohammad Aeman Kazali: Data curation, Methodology, Software, Writing – original draft

Conflicts of Interest

The authors declare no conflicts of interest.

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