DOI: 10.69946/ojms/2025.03.02.08, pp(70-78)

Association of *VDR* gene polymorphism (rs2228570 A>C, G, T) with risk of Benign Prostatic Hyperplasia in Iraqi patients

Basim Mohammed Abdul Latif [1], Ammar Ahmed Sultan[2], Thikra Ata Ibrahim[3]
[1,2,3] Department of Biology, College of Education for Pure Sciences, University of Diyala, Baqubah, 32001, Iraq
*Corresponding Author: Basim Mohammed Abdul Latif Email :basimalhusani@gmail.com

Abstract
Benign prostatic hyperplasia (BPH) represents a prevalent urological condition in aging men, characterized by prostate enlargement as well as lower urinary tract symptoms. We investigated the link between the *VDR* gene variant rs2228570 (A>C, G, T) and the risk for BPH in Iraqi patients. A total of 3 patients with BPH, as well as 10 healthy individuals (50–70 years) recruited in Baqubah Teaching Hospital, Diyala, participated in this investigation. The genomic DNA was extracted and amplified by PCR, and then sequenced by Geneious software. Three genotypes (AA, AG, GG) were found. We found a significant association between genotype distribution and risk of BPH. The GG genotype and G allele were more common in the controls with a significant protective effect (P = 0.001; OR = 29.00). Conversely, the AA genotype and A allele were common in the patients with a significant risk factor (P = 0.026). No significant effect was identified for the AG genotype (P = 0.862). Hardy–Weinberg testing revealed equilibrium for both populations without any evidence for genetic instability. In sum, these findings indicate that the *VDR* gene rs2228570 can be involved in the susceptibility towards BPH, underscoring its value in terms of testing genetic risk as well as preventing its occurrences.

Keywords: VDR, BPH, rs2228570, Single nucleotide polymorphism, Hardy–Weinberg equilibrium

1. Introduction

The prostate is a component of the male reproductive system responsible for storing and secreting seminal fluid in adult men. A typical prostate measure approximately three centimeters in length and weighs around twenty grams [1]. It is located in the pelvic cavity beneath the urinary bladder and anterior to the rectum, surrounding a portion of the urethra, the tube that carries urine from the bladder during urination and seminal fluid during ejaculation [2]. The prostate commonly enlarges with age, and such enlargement can obstruct urinary outflow from the bladder, potentially leading to complications in the bladder, urinary tract, or kidneys [3].

The prostate is filled with many tiny glands, which contribute about 20 percent of the seminal fluid, as well as housing male sex hormones or androgens [4]. These include testosterone, synthesized in the testes, and dehydroepiandrosterone (DHEA), produced by the adrenal glands, which is subsequently converted into testosterone by the prostatic enzyme 5α-reductase [5]. Anatomically, the adult prostate is divided into four distinct zones. The first is the Peripheral Zone (PZ), located primarily on the posterior and lateral aspects of the gland, extending variably to the apex and anteriorly. It surrounds the Central Zone (CZ), opening its ducts into the distal prostatic urethra. The peripheral zone contains the majority (approximately 70%) of glandular tissue in a

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normal prostate and represents the most frequent site of prostate cancer (PC) development, making it highly susceptible to inflammation as well [6].

The second zone, the Central Zone (CZ), comprises about 25% of the glandular tissue and demonstrates resistance to both cancer and inflammation relative to other regions. Cells here are found in these areas show different morphologies, such as an enlarged cytoplasm and also slightly. Their appearance includes basophilic properties and bigger nuclei [7]. The third comes to be known as the Transitional Zone (TZ), made up of slightly ducts and shorter glandular lobules with few mucosal glands. The overhang transitional zone extends below the assemblage and arises out of a sequence of patterns, culminating in the fascination with the assemblage. The overhang is an overhang of other appearances below the assemblage, and is a consequence of a sequence of appearances, culminating in the fetishism of the assemblage [8]. An amorphous zone of collagen usually separates it; however, more evident in the presence of younger adults' post-puberty, structural and histological differences within the transitional zone are not well defined. This zone is the exclusive site of BPH in elderly men [9]. Parenchymal cells in this region frequently undergo extensive proliferation, forming nodular epithelial clusters, which can compress the adjacent prostatic urethra and result in urinary difficulties, a condition referred to as benign prostatic hyperplasia [10].

The fourth zone is the periurethral zone, containing mucosal and submucosal glands, which may also undergo pathological growth in later stages of BPH. Expansion of this zone, particularly of connective tissue and nodular glandular elements in the transitional zone, increases urethral pressure and leads to further bladder urine retention [11]. BPH induced urethral compression can cause bladder wall thickening, potentially weaken the bladder and impair complete emptying [12], ultimately resulting in urinary retention. Several mechanisms have been proposed for BPH development, including an age-related imbalance between estrogen and testosterone [13]. Prostate enlargement can progress to malignancy, affecting the urinary and reproductive systems and potentially spreading to adjacent or distant organs, which necessitates aggressive treatment for patient survival [14].

The Vitamin D receptor (VDR) is considered one of the most important regulators of the body's response to vitamin D. It functions as a nuclear transcription factor that binds to the active form of vitamin D, 1,25-$(OH)_2D_3$, to regulate gene expression associated with calcium balance and immunity [15]. This gene is located on chromosome 12 at position q13.11 and is expressed in many tissues, including the intestine, bone, kidney, immune system tissues, and the prostate. Polymorphisms in the VDR gene have been found to be associated with genetic susceptibility to several diseases, including osteoporosis, immune disorders, and cancers [16].

The role of the Vitamin D receptor (VDR) in benign prostatic hyperplasia (BPH) is that it influences the gene expression of a group of genes involved in regulating cell growth and differentiation in the prostate. Vitamin D can reduce the proliferation rates of cancer cells and benign prostatic hyperplasia (BPH) [17].

The Vitamin D receptor (VDR) can also affect various hormonal pathways that play a role in prostate growth, including testosterone and dihydrotestosterone (DHT). The potential mechanism involves the effect of vitamin D on specific receptors in prostate cells, which helps reduce abnormal

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cell proliferation and limit chronic inflammation, a contributing factor in the development of BPH [18].

Objectives of the Study:

1. In order to search for a link between the rs2228570 VDR gene polymorphism and benign prostatic hyperplasia (BPH) risk.
2. In an endeavor to contrast genotype (AA, AG, GG) as well as allele (A, G) frequency in healthy controls versus BPH cases.
3. To determine the effect of the variant rs2228570 as a risk or protective factor for BPH.
4. To determine Hardy–Weinberg equilibrium for both populations in order to create genetic stability for the entire study population.

2. Materials and Methods

The study was conducted at the Molecular Genetics Laboratory, College of Education for Pure Sciences, University of Diyala, Iraq. It included patients with benign prostatic hyperplasia (BPH) and healthy individuals, all of Iraqi origin residing in Diyala Governorate, who were recruited at Baqubah Teaching Hospital. Blood samples were collected from the participants between October 2023 and May 2024.

The single-nucleotide polymorphism (SNP) of the VDR gene at rs2228570 was analyzed using the polymerase chain reaction (PCR) technique. Primers were being worked out by performing on the NCBI Primer-BLAST tool. The forward primer sequence was GAGGGCTCACCTGAAGAAGC, and the reverse primer sequence was CTGACTCTGGCTCTGACCGT. The expected PCR product length was 200 base pairs, with GC content of 60.39% for the forward primer and 61.25% for the reverse primer. The melting temperatures were 60.00°C and 60.00°C, respectively. The PCR reaction mixture consisted of primers, DNA template, ready-to-use master mix, and nuclease-free water, with a final volume of 25 µl. Amplification success was confirmed by electrophoresis on an agarose gel.

Each sample reaction was prepared with a total volume of 25 µl. Samples from healthy individuals and BPH patients were combined with the reaction mixture and subjected to thermal cycling under the following conditions: initial denaturation at 94°C for 5 minutes; denaturation at 94°C for 30 seconds; primer annealing at 60°C for 30 seconds; extension at 72°C for 5 minutes; followed by a final extension at 72°C for 5 minutes. These steps were repeated for 35 cycles. Post-amplification, PCR products were resolved on a 1% agarose gel at 90 V for 1.5 hours. The amplified DNA was then sent to Macrogen Company (South Korea) for Sanger sequencing of the VDR gene. Genotypes were classified according to Hardy–Weinberg equilibrium as potential protective or risk factors based on nucleotide sequence analysis using Geneious software.

3. Results

The *VDR* gene was successfully amplified in the coding region at the rs2228570 site. The A>C, G, T polymorphism has been studied in patients with benign prostatic hyperplasia (BPH) and healthy controls. The results indicated that *VDR* gene fragment amplification at the coding area of rs2228570 produced a molecular band of approximately 200 base pairs in patients and normal samples. This

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was also confirmed by ethidium bromide staining and visualization under ultraviolet (UV) light, as presented in Fig. 1.

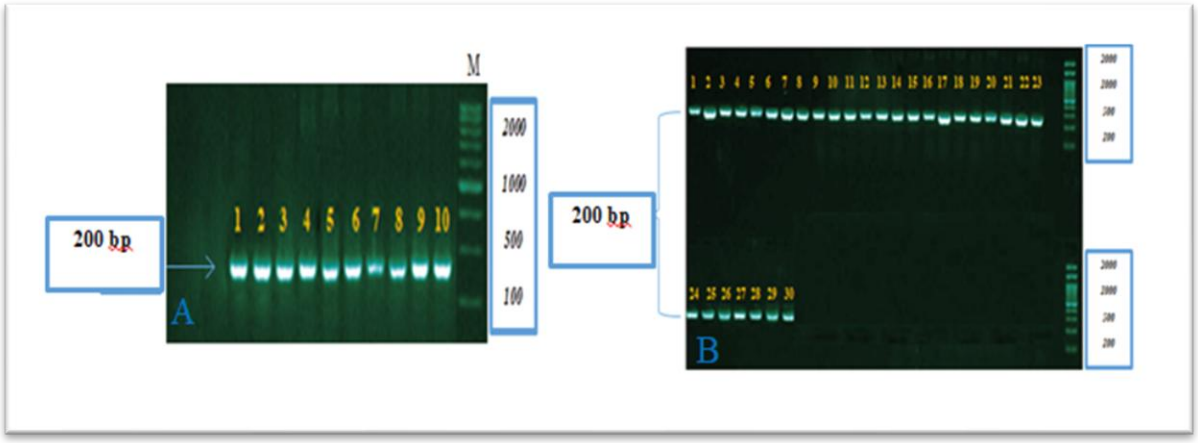

Fig. 1 PCR products of the coding region in the VDR gene from healthy controls and patients with benign prostatic hyperplasia (BPH). The PCR products were separated on a 1.5% agarose gel at 90V for 1.5 hours, stained with ethidium bromide, and visualized under UV light.

3.1 Comparison of Genetic Polymorphism in the rs2228570 A>C, G, T Locus of the VDR Gene Between Study Samples and Reference Gene Bank

The nucleotide sequence of all the study samples, including 30 BPH patient samples and 10 Healthy control samples, was charted together in a single graph, shown in Fig. 2, to identify expression variations in the amplified segment of the VDR gene on chromosome 10. This fragment, with a molecular weight of 200 base pairs, includes the rs2228570 A>C, G, T polymorphism. Sequence alignment and analysis were performed using Geneious software (National Center for Biotechnology Information, NCBI). The alignment method was employed to determine the exact nucleotide variations and their positions. Subsequently, all sample sequences were compared with each other and with the reference DNA sequence documented in the NCBI database. Comparison of the nucleotide sequences revealed the presence of point mutations of the transition type in BPH patient samples relative to both the reference DNA sequence and the sequences of healthy controls, as confirmed using the NCBI online database.

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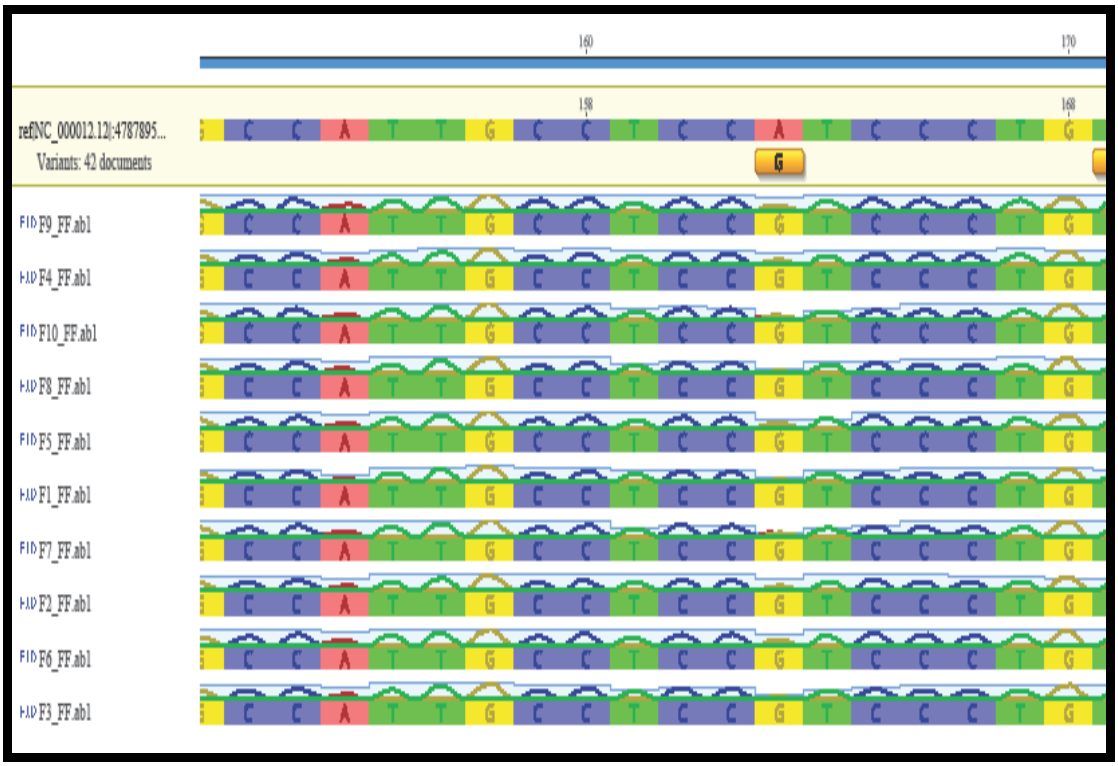

Fig. 2 illustrates the comparison of nucleotide sequence alignments for a fragment of the *VDR* gene among several BPH patient samples, healthy controls, and the reference gene bank sample. The figure highlights the location of the rs2228570 polymorphism and indicates the type of mutation identified (NCBI, 2024).

3.2 Genotype Determination of the *VDR* Gene at the rs2228570 A>C,G,T Locus

The results presented in Table 1 indicate that the homozygous GG genotype was observed in only one BPH patient (3.33%), whereas it appeared in 50% of the healthy control group, indicating a marked reduction among patients. According to Fisher's exact test (P = 0.001), this difference is statistically significant. The GG genotype is considered a strong risk factor against the disease, with an odds ratio (OR) of 29.00 within a 95% confidence interval (CI: 2.87–708.14).

The heterozygous AG genotype was the most prevalent in both groups, appearing in 60% of BPH patients and 50% of healthy controls. Fisher's exact test (P = 0.862) indicates no significant difference, with an OR of 1.06 (95% CI: 0.24–4.55), suggesting that the AG genotype shows a significant effect as a risk factor for the disease. The homozygous AA genotype was absent in the healthy controls (0.00%) but appeared in 36.66% of BPH patients. This difference was statistically significant (P = 0.026) and the AA genotype is considered a risk factor, with an OR of 0.00 (95% CI: 0.0000–0.7435).

Allelic frequency analysis showed that the G allele appeared at 75% in healthy controls versus 33.33% in patients, indicating a significant decrease in patients and confirming its role as a risk allele (OR = 6.00; 95% CI: 1.91–20.32). Conversely, the A allele was higher in patients (66.67%) compared to controls (25%), suggesting it acts as a protective allele (OR = 0.17; 95% CI: 0.05–0.52). Hardy–Weinberg equilibrium analysis in Tables 1 and 2 demonstrated that the distribution of the three genotypes (AA, AG, GG) and allelic frequencies of the VDR gene at rs2228570 were in relative equilibrium. For healthy controls, P = 0.291, indicating equilibrium. For patients, P =

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0.0552, which is close to the significance threshold but not statistically significant, indicating a quasi-equilibrium. This indicates no significant deviation between observed and expected genotype and allele frequencies in both groups, reflecting relative genetic stability in the study population.

Table 1: Assessment of the Relationship Between *VDR* Genotypes and Alleles at the rs2228570 A>C, G, T Locus in the Study Groups

Genotype // rs2228570 A>C, G, T	**Control No. (%)**	**Patients No. (%)**	**Fisher's/P-value**	**O.R. (C.I.)**
AA	0 (0.00%)	11 (36.66%)	0.026*	0.00 (0.0000 - 0.7435)
AG	5 (50%)	18 (60%)	0.862 NS	1.06 (0.24 - 4.55)
GG	5 (50%)	1 (3.33%)	0.001 *	29.00 (2.87 - 708.14)
Total	10 (100%)	30 (100%)		
Allele	*Frequency*			
A	5 (25%)	40 (66.67%)	O.R. (C.I.) = 0.17 (0.05 - 0.52)	
G	15 (75%)	20 (33.33%)	O.R. (C.I.) = 6.00 (1.91 - 20.32)	
NS: Non-Significant, * (P≤0.05)				

Table 2: Distribution of Genotypes and Allelic Frequencies of the VDR Gene at the rs2228570 A>C, G, T Locus in Study Groups According to Hardy–Weinberg Equilibrium

Hardy P-values	**Allele frequencies**		**Genotype // rs2228570 A>C, G,T**				**Grouper**
0.2918 NS	G	A	GG	AG	AA	No.	Observed	Control 10
	15	5	5	5	0.00			
	75	25	50	50	0.00	%		
	Not diagnosed		5.63	3.75	0.63	No.	Expected	
			56.25	37.5	6.25	%		
0.0552*	20	40	1	18	11	No.	Observed	Patients 30
	33.33	66.67	3.33	60	36.66	%		
	Not diagnosed		3.33	13.33	13.33	No.	Expected	
			11.11	44.44	44.44	%		

4. Discussion

This study was conducted to investigate the association between the rs2228570 A>C, G, T polymorphism in the VDR gene and susceptibility to benign prostatic hyperplasia (BPH), comparing BPH patients with a healthy control group. The genotypic and allelic distributions are summarized in Tables 1 and 2. The results revealed that the GG homozygous genotype was present in only one patient (3.33%) but in five individuals (50%) of the control group, indicating a significant reduction

DOI: 10.69946/ojms/2025.03.02.08, pp(70-78)

among patients (Fisher's P = 0.001). The GG genotype exhibited a strong risk factor against BPH, with an odds ratio (OR) = 29.00 (95% CI: 2.87–708.14).

The heterozygous AG genotype was the most frequent in both groups, observed in 60% of patients and 50% of controls, with no significant difference (P = 0.862, OR = 1.06, 95% CI: 0.24–4.55), indicating a limited or neutral effect on disease susceptibility. Conversely, the AA homozygous genotype was absent in controls (0%) but present in 36.66% of patients, showing a significant association with increased BPH risk (P = 0.026, OR = 0.00, 95% CI: 0.0000–0.7435). Allelic analysis further confirmed these findings, with the G allele being more frequent in controls (75%) versus patients (33.33%), indicating a risk role (OR = 6.00, 95% CI: 1.91–20.32), while the A allele was elevated in patients (66.67%) compared to controls (25%), identifying it as a protective allele (OR = 0.17, 95% CI: 0.05–0.52).

Hardy–Weinberg equilibrium analysis demonstrated that genotype and allele distributions were relatively balanced in controls (P = 0.291) and nearly balanced in patients (P = 0.0552), indicating no significant deviation from expected frequencies, which suggests relative genetic stability in the study population. These results are consistent with previous meta-analyses, including a study of 11 samples ,which concluded that the rs2228570 polymorphism in the VDR gene is significantly associated with the risk of BPH. Specifically, the G allele was identified as a protective factor, particularly among Caucasians, whereas the A allele increased susceptibility to the disease [19]. reported that carriers of the A allele showed reduced transcriptional activity of the vitamin D receptor, resulting in impaired regulation of prostate cell growth and increased hyperplasia markers, which aligns with our findings of elevated AA genotype frequency in patients. Furthermore, demonstrated that the rs2228570 polymorphism affects VDR binding efficiency to regulatory genomic sites, altering cellular responsiveness to the active form of vitamin D, $1,25(OH)_2D_3$, which may explain the observed biological effect of the A allele in increasing BPH risk [20].

However, partial discrepancies exist with the study by which did not find a strong association between rs2228570 and BPH in certain Asian populations. These differences may reflect environmental factors, sunlight exposure, and dietary habits that influence vitamin D status, highlighting the importance of geographic and ethnic context in interpreting the association between VDR polymorphisms and BPH risk [21]. The findings of the current study showed partial inconsistency with those reported who found no significant association between the rs2228570 variant and benign prostatic hyperplasia (BPH) in certain Asian populations [22], attributing this discrepancy to environmental and dietary factors as well as differences in sun exposure. Similarly reported no significant association of the same variant with the disease ($p > 0.05$) was reported, whereas the rs1544410 variant demonstrated a strong association with increased risk, particularly in the G/G genotype [23].

The findings of the current investigation agree with the majority of the previous studies, highlighting the significant role of the VDR gene in the susceptibility towards or the protection against BPH despite a few ethnic as well as environmental divergences. In consonance with this finding, the association found between the variant rs2228570 and the risk for BPH upholds its postulation as a gene with significance, necessitating larger-scale research for its validation. Together, the current study demonstrates that VDR gene polymorphism at rs2228570 is actually has a very important critical effect on the susceptibility to BPH, as the GG genotype and G allele were

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risk factors ones. whereas the AA genotype and A allele put someone at elevated risk of the disease. To a large extent, such findings tend to parallel others, with earlier studies, suggesting the crucial region of VDR prostate physiology and the pathogenesis of BPH. However, the differences between populations justify multicenter studies Using bigger sample groups to explain this genetic association fully.

5. Conclusion

The polymorphism of the vitamin D receptor (*VDR*) gene at the rs2228570 locus is strongly associated with the risk of benign prostatic hyperplasia (BPH) in a sample of the Iraqi population. The GG genotype and the G allele were found to be prevalent among patients and considered as risk factors for the disease, whereas the AA genotype and the A allele were absent in healthy controls and rare among patients, and were regarded as completely protective against the disease.

Conflict of Interest:
According to the authors, conflict of interest does not exist.

Funding:
This was a study that did not consume any funds..

Ethical Approval:
In the University of Diyala, the author submitted to the College of Education.
Ethical approval for the study was obtained. The Pure Sciences -Baqubah, Iraq..

Acknowledgments:
To everyone at Baqubah Teaching Hospital / Diyala, we would like to say Thank You .We would like to express our gratitude to the Health Directorate / Diyala in their effort to be helpful and useful and they do not hesitate to help us .assisted us in sample and data collection.

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