Original Article

CYP3A4 Gene with Risk of Benign Prostatic Hyperplasia

# Association of the CYP3A4 Gene (rs2740574 C>A, G, T) with the Risk of Benign Prostatic Hyperplasia in Iraqi Patients

**Basim Mohammed Abdul Latif, Ammar Ahmed Sultan and Thikra Ata Ibrahim**

## ABSTRACT

**Objective:** To evaluate the association between the genetic polymorphism rs2740574 in the CYP3A4 gene and the risk of developing benign prostatic hyperplasia in a sample of Iraqi patients.
**Study Design:** Descriptive study
**Place and Duration of Study:** This study was conducted at the Baqubah Teaching Hospital, Diyala Province, Iraq from October 2023 and May 2024.
**Methods:** This descriptive study included 30 benign prostatic hyperplasia patients and 10 healthy controls aged between 50 and 70 years. DNA was extracted from the samples, and the target fragment was amplified using polymerase chain reaction. Genetic variations were analyzed using the Geneious software. The sequencing analysis of the rs2740574 polymorphism in the CYP3A4 gene revealed three genotypes: CC, CT, and TT.
**Results:** A strong association between the genotype distribution of this polymorphism and the risk of BPH. The CC genotype and C allele were highly prevalent among the control group and represented a strong protective factor against BPH, supported by Fisher's exact test (P=0.000) and an odds ratio (OR) of 0.003. In contrast, the CT genotype was significantly more frequent in patients and indicated a very high risk of disease (P=0.000, OR=81.00). The T allele was present in 51.67% of patients versus only 5% in controls, making it a major risk factor with an OR of 20.31. The TT genotype was rare and not statistically significant but was still considered a potential risk factor according to Fisher's test (P=0.721) and OR=1.84. The Hardy–Weinberg equilibrium test showed genetic balance in the control group (P = 0.8678, not significant), indicating a normal distribution unaffected by disease pressure. However, the patient group showed a clear deviation from equilibrium (P=0.000), reflecting the influence of the disease state on genotype distribution in this sample.
**Conclusion:** The genetic polymorphism of the rs2740574 of the gene could lead to the incidence of autism. A case of benign is that CYP3A4 gene has a huge role in genetic predisposition to benign. Women: prostatic hyperplasia.
**Key Words:** CYP3A4, Benign prostatic hyperplasia, Single neuclotide polymorphism, Hardy–Weinberg

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## INTRODUCTION

The prostate is part of the male reproductive system, storing and secreting seminal fluid. It typically measures about three centimeters in length and weighs twenty grams.[1] In the pelvis beneath the bladder and in front of the rectum, it surrounds part of the urethra, which carries urine and semen.[2] With age, the prostate enlarges, potentially obstructing urinary flow and causing complications in the bladder, urinary tract, or kidneys.[3]

The prostate's small glands produce about 20% of seminal fluid and are regulated by androgens, mainly testosterone synthesized in the testes and derived from dehydroepiandrosterone (DHEA) from the adrenal glands. DHEA is converted to testosterone by 5α-reductase in the prostate.[4]

The adult prostate has four zones. The peripheral zone (PZ), mainly posterior and lateral, accounts for 70% of glandular tissue, is the most frequent origin of prostate cancer, and is prone to inflammation.[5]

The central zone (CZ) forms 25% of glandular tissue, is more resistant to cancer and inflammation, and contains cells with thick cytoplasm, basophilic staining, and large nuclei.[6]

The transitional zone (TZ) contains short ducts and mucous glands, bounded by collagenous tissue.

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After puberty, its glands lack clear definition, but in older men it enlarges in nodular masses of epithelial cells, causing urinary obstruction characteristic of BPH.[3,7]

The periurethral zone includes mucous and submucous glands, which may proliferate during BPH, adding urethral pressure and urinary retention.[5]

Benign prostatic hyperplasia compresses the urethra, thickening and weakening the bladder, leading to incomplete emptying and urinary retention.[8] Proposed causes include hormonal imbalance (estrogen/testosterone). BPH may predispose to prostate cancer in older men, affecting urinary and reproductive systems, with possible metastasis requiring proper management.[9,10]

Polymorphisms in cytochrome P450 genes, such as CYP17A1 and CYP3A4, influence testosterone metabolism and prostate growth, linking them to prostate cancer risk.[11]

CYP3A4 encodes a major enzyme metabolizing endogenous and exogenous compounds, mainly in the liver and intestines, and affects drug metabolism and therapeutic responses.[12,13] Variants, including rs2740574, alter enzyme activity, influencing susceptibility to diseases like BPH and treatment outcomes.[14] Studying these variants enhances understanding of drug response variability and supports personalized medicine.[15]

This study investigates the association between CYP3A4 rs2740574 polymorphism and BPH risk in Iraqi patients, aiming to evaluate its role as a predictive biomarker of disease susceptibility.

# METHODS

This research was conducted at the Molecular Genetics Laboratory, College of Education for Pure Sciences, University of Diyala, Iraq. It focused on BPH patients and healthy Iraqi individuals from Diyala province, admitted to Baqubah Teaching Hospital. Blood samples were collected in October 2023 and May 2024.

Genetic polymorphism of the CYP3A4 gene (rs2740574) was analyzed using PCR. Primers were designed with NCBI Primer-BLAST (Forward: CACACCACTCACTGACCTCC, Reverse: GTAGGTGTGGCTTGTTGGGA). The expected product length was 217 bp, with GC contents of 60% and 55%, and melting temperatures of 59.97°C and 59.89°C, respectively. PCR mixtures contained primers, DNA template, master mix, and nuclease-free water in 25 µl volume. Amplification was confirmed by agarose gel electrophoresis.

Thermal cycling included: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 5 min; followed by a final extension at 72°C for 5 min. PCR products were separated on 1% agarose gel at 90 V for 1.5 h. Amplified DNA was sequenced at Macrogen (South Korea) using the Sanger method. Genotypes were classified as protective or causal based on nucleotide sequence analysis with Geneious software, applying Hardy–Weinberg equilibrium.

Quality control included duplicate PCR runs, positive/negative controls, and chromatogram evaluation of sequencing. Ethical approval was obtained from the Institutional Ethics Committee, University of Diyala, and informed consent was provided by all participants, in accordance with the Declaration of Helsinki.

# RESULTS

The results of amplifying a segment of the CYP3A4 gene promoter region at the rs2740574 polymorphic site showed that the molecular weight of the resulting bands was 217 base pairs for both patient and control samples after staining with ethidium bromide and visualization under ultraviolet light (Fig. 1).

The nucleotide sequences of all samples subjected to sequencing30 samples from patients with benign prostatic hyperplasia (BPH) and 10 samples from healthy individuals were aligned and compared using a single chart (Fig. 2). The aim was to investigate genetic variations within the amplified region of the CYP3A4 gene, located on chromosome 10, with a molecular size of 217 base pairs, and encompassing the polymorphic site rs2740574. The analysis was conducted using the Geneious software from the National Center for Biotechnology Information (NCBI). Multiple sequence alignment was employed to identify nucleotide substitutions and their precise locations. These sequences were then compared with one another as well as with the reference DNA sequence available. The results revealed the presence of point mutations of the transition type in the BPH patient samples when compared to both the reference DNA sequence and the sequences of the healthy control group, as documented on the NCBI platform.

The genotypic distribution results revealed that the homozygous CC genotype was the most prevalent among the healthy control group, with 9 out of 10 individuals (90%) carrying this genotype. The C allele frequency in this group was 19 (95%). In contrast, only one patient out of 30 with benign prostatic hyperplasia (BPH) exhibited the CC genotype (3.33%), with a corresponding C allele frequency of 29 (48.33%). According to Fisher's exact test, this difference was statistically highly significant (P=0.000), and the odds ratio (OR) was 0.003 with a 95% confidence interval (CI) of 0.000–0.067. These findings suggest that the CC genotype and C allele may serve as strong protective factors against BPH (Table 1).

On the other hand, the CT heterozygous genotype was found in 27 out of 30 patients (90%) compared to only 1 case (10%) in the control group. The associated OR was 81.00, with a 95% CI ranging from 7.76 to

1834.07, and the result was statistically significant (P = 0.000), indicating that the CT genotype represents a very high risk factor for BPH. The TT homozygous genotype was identified in 2 patients (6.66%) and was absent in the control group.

**Table No. 1: Evaluation of the Association between Genotypes and Alleles of the CYP3A4 Gene at the rs2740574 (C>A, G, T) Polymorphic Site Across Study Groups**

| Genotype //rs2740574 C>A,G,T | Control | Patients | Fisher's/P-value | O.R. (C.I.) |
|---|---|---|---|---|
| CC | 9 (90%) | 1 (3.33%) | 0.00* | 0.003<br>(0.000 - 0.067) |
| CT | 1 (10%) | 27 (90%) | 0.00* | 81<br>(7.76 - 1834.07) |
| TT | - | 2 (6.66%) | 0.721 NS | 1.84<br>(0.09 - 36.60) |
| Total | 10 (100%) | 30 (100%) | | |
| **Allele** | **Frequency** | | | |
| C | 19 (95%) | 29 (48.33%) | O.R. (C.I.) = 0.05 (0.00 - 0.30) | |
| T | 1 (5%) | 31 (51.67%) | O.R. (C.I.) = 20.31 (2.69 - 153.61) | |
| *P≤0.05 (Significant), NS: Non-Significant. | | | | |

**Table No. 2: Distribution of Genotypes and Allelic Frequencies of the CYP3A4 Gene at the rs2740574 (C>A, G, T) Polymorphic Site in Study Groups According to the Hardy-Weinberg Equilibrium in Benign Prostatic Hyperplasia Compared to the Control Group**

| **Group** | | **Genotype // rs2740574 C>A,G,T** | | | **Allele frequencies** | | **P value** |
|---|---|---|---|---|---|---|---|
| | | **CC** | **CT** | **TT** | **C** | **T** | |
| Patients | Observed | 9 | 1 | - | 19 | 1 | 0.867 (NS) |
| | | 90% | 10% | - | 95 | 5 | |
| | Expected | 9.03 | 0.95 | 0.03 | Not diagnosed | | |
| | | 90.25 | 9.5 | 0.25 | | | |
| Controls | Observed | 1 | 27 | 2 | 29 | 31 | $0.000^{*}$ |
| | | 3.33% | 90% | 6.66% | 48.34% | 51.67% | |
| | Expected | 7.01 | 14.98 | 8.01 | Not Diagnosed | | |
| | | 23.36 | 49.94 | 25.69 | | | |

NS = Not significant *P≤0.01 (Significant)

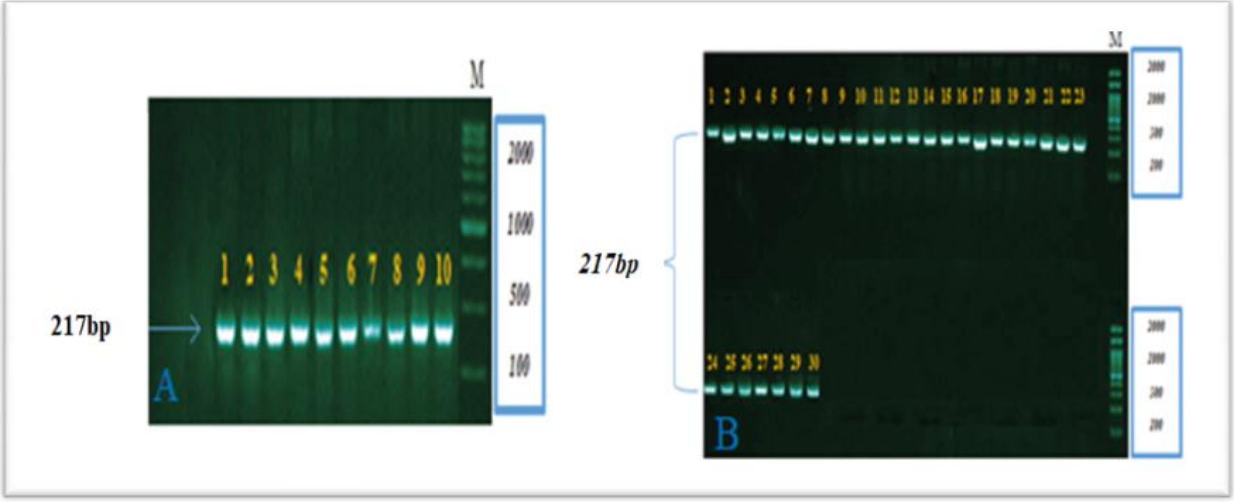

**Figure No. 1: The amplification result of a segment from the promoter region of the CYP3A4 gene at the rs2740574 C>A, G, T polymorphic site in (A) the healthy control group and (B) patients with benign prostatic hyperplasia**

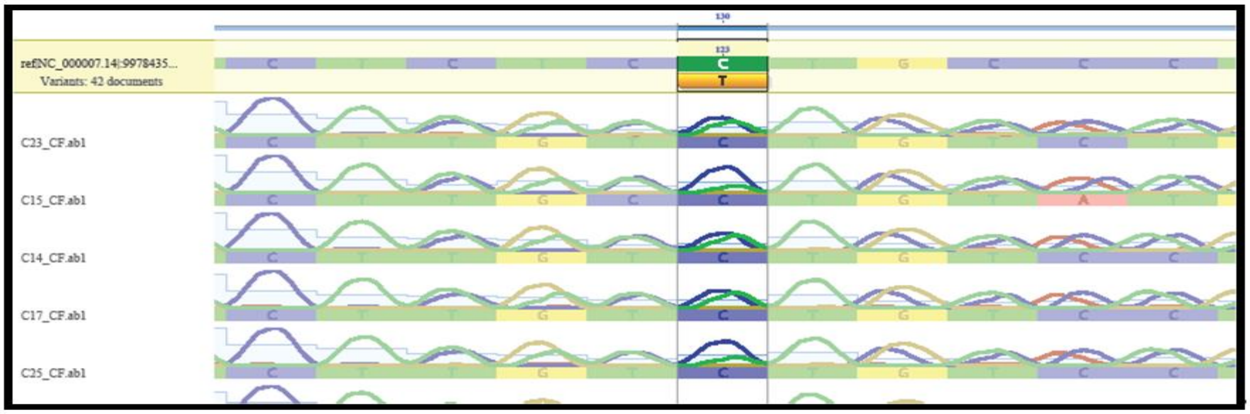

**Figure No. 2: Comparative Alignment of Nucleotide Sequences for a Segment of the CYP3A4Gene Among BPH Patient Samples, Healthy Controls, and the GenBank Reference Sequence. This figure illustrates the position of the rs2740574 (C>A, G, T) polymorphism and identifies the type of mutation observed. The alignment was performed using data from NCBI (2024)**

The corresponding P-value was 0.721, and the OR was 1.84 with a 95% CI of 0.09–36.60, suggesting a potential risk associated with this genotype, albeit not statistically significant. Regarding overall allele distribution, the T allele was significantly more common among patients (51.67%) compared to only 5% in healthy individuals. This difference was also statistically significant, with an OR = 20.31 and a 95% CI of 0.00–0.30, indicating that the T allele is a major genetic risk factor for developing BPH. Additionally, the Hardy-Weinberg equilibrium analysis, shown in Table (2), demonstrated that the control group was in genetic equilibrium (P = 0.8678; NS), suggesting that genotype frequencies followed expected Mendelian distribution and were unaffected by disease pressure. In contrast, the patient group exhibited a significant deviation from equilibrium (P = 0.000), possibly due to disease-related selective pressure or a direct association between the genotypes at the studied locus and susceptibility to BPH (Table 2).

## DISCUSSION

This study was conducted based on a comparative analysis between two groups: patients diagnosed with benign prostatic hyperplasia (BPH) and a healthy control group, to investigate the genetic polymorphism rs2740574 (C>A, G, T) in the CYP3A4 gene. The results of the genotypic distribution presented in Tables (1–2) revealed that the CC homozygous genotype was the most prevalent in the healthy group, with 90% of individuals carrying this genotype, and a C allele frequency of 95%. In contrast, this genotype was observed in only one BPH patient (3.33%), with a corresponding C allele frequency of 48.33%. These differences were statistically significant (P=0.000, OR=0.003), suggesting that both the CC genotype and C allele may serve as protective factors against BPH. Conversely, the CT heterozygous genotype was found in 90% of the BPH patients, compared to only 10% in the control group (OR=81.00, P=0.000), indicating a strong association with increased risk of developing BPH. The TT homozygous genotype was rare, appearing in only 6.66% of patients and not at all in controls, with a non-significant (P=0.721, OR=1.84), suggesting a limited impact due to its low frequency. In terms of allelic distribution, the T allele was markedly more frequent among patients (51.67%) compared to only 5% in controls, with strong statistical significance (OR=20.31), identifying it as a potential risk allele. The Hardy–Weinberg equilibrium test showed a balanced genotypic distribution in the control group (P=0.8678), suggesting a normal, unaffected population. In contrast, there was a significant deviation from equilibrium in the BPH group (P=0.000), possibly reflecting disease-associated genetic pressure or a direct genotype–phenotype correlation.

This polymorphism resides in the promoter of CYP3A4 gene and is synonymous with the -392A>G mutation that is often written CYP3A4*1B. This variant is termed to influence the gene expression of the responsible enzyme to metabolise testosterone to less active forms. In the past, such a change has occurred. It has been implicated to have both knock-on and malignant prostate states groups of population.[15,16]

To prove this point, one study done in Korea illustrated a strong relationship between specific variant forms of CYP3A4, especially CYP3A4-Ht-2 and higher metastatic potential within prostate cancer cells.[17] Also, a recent research article emphasized that CYP3A4*1B (rs2740574) is one of the most commonly investigated polymorphisms regarding relation to prostate-related disorders both with respect to BPH and prostate cancer.[18,19]

In addition, a study done in South Africa indicated that the G allele of rs2740574 was associated with susceptibility to prostate cancer in both Blacks and

White men, having emphasized the significance of this polymorphism in ethnically various ethnics groups.[20]

The research suggests that additional research into bigger and wider research is necessary. This is done by way of populations to confirm such findings and to understand more into the molecular mechanisms through which this genetic variation leads to development of this disease.

# CONCLUSION

The genetic polymorphism of the rs2740574 of the gene could lead to the incidence of autism. A case of benign is that CYP3A4 gene has a huge role in genetic predisposition to benign. Prostatic hyperplasia women have T allele is linked with a higher chance of getting BPH and the C allele seems to have an effect of suppressing BPH protective effect. The results outline the possible relevance of genetic screening of finding people who are more risky to BPH and opening the way to the potential use this polymorphism as a prospective biomarker of early diagnosis and disease prevention.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design or acquisition of analysis or interpretation of data: | Basim Mohammed Abdul Latif, Ammar Ahmed Sultan |
| Drafting or Revising Critically: | Basim Mohammed Abdul Latif, Thikra Ata Ibrahim |
| Final Approval of version: | All the above authors |
| Agreement to accountable for all aspects of work: | All the above authors |

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