ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Association of -34 T > C CYP17 Gene Polymorphism with Benign Prostatic Hyperplasia in Babylon Province, Iraq

Rawaa G. Abdullah, Abdulsamie H. Altaee*, Mohammed R. Joodi

College of Medicine, University of Babylon, P. Code 51002, Hilla PO Box 473, Iraq.

***Corresponding Author Email:** *abdulsamie68@gmail.com*

Abstract

Benign prostatic hyperplasia (BPH) is the most common benign tumor in men, and its incidence is age-related. This study aims to investigate the effect of -34 T > C CYP17 gene polymorphism on BPH susceptibility and clinical progress.Fifty males patients with BPH clinically diagnosis attended to Al-Hillah Teaching Hospital, as well as fifty males apparently healthy control in a case-control study were subjected to present study. The -34 T > C CYP17 gene polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.Genetic analysis of -34 T > C CYP17 gene polymorphism shows a significantly higher difference when comparing patients with the control groups. The dominant and codominant models of -34 T > C CYP17 gene polymorphism were shown significant change when compared with healthy control The T/C-T/T is genotyping responsible and related to the risk of BPH.In conclusion, -34 T > C CYP17 gene polymorphism could have an important role in the development and pathogenesis of BPH.

Kewords: *Benign prostatic hyperplasia, CYP17 gene polymorphism, PCR-RFLP, Babylon, Iraq.*

Introduction

Benign prostatic hyperplasia (BPH) is one of the main findings influencing men of expanding age. By age 50 years, about 50% of men are diagnosed with BPH; by 80 years, 90% of men are diagnosed, and thegreatest prevalence occurs among men ages 70 to 79 years[1,2]. Normal risk factors for BPH incorporate expanding metabolic syndrome, age, family history of BPH, functioning testicles, history of diabetes, obesity, and black race [2,3]. Smoking, diet, and exercise can influence BPH predisposition [2,4,5].

Male hormones or androgens assume in all likelihood a part in prostate development. The most critical androgen is testosterone, which is delivered all through a man's lifetime. The prostate proselytes testosterone to an all the more effective androgen, dihydrotestosterone (DHT) [6] invigorates cell development in the tissue that lines the prostate organ (the glandular epithelium) and is the significant reason for the quick prostate expansion that happens amongst adolescence and youthful adulthood [7]. DHT is a prime suspect in prostate development in later adulthood [7].

Estrogens or the female sex hormone may likewise assume a part in BPH [8], however, some estrogen is constantly present in men. As men age, testosterone levels drop and the extent of estrogen increments, conceivably activating prostate development [9].

(CYP17) gen is situated on chromosome 10q24.3, traverses 6569 bp and is partitioned into eight exons, encodes the chemical cytochrome P-450C17, which capacities at entering branch focuses in steroid hormone biosynthesis in the adrenal organ, ovary, and gonads [10].

Cytochrome P-450C17 intercedes both steroid 17 hydroxylase action, which changes over pregnenolone to dehydroepiandrosterone, and 17,20-lyase movement, which creates androstenedione from progesterone, antecedents of testosterone and estrogen.

These androgens may then be changed over to estrone, testosterone, and estradiol [11]. The current study aims to investigate the effect of -34 T > C CYP17 gene polymorphism on BPH susceptibility and clinical progress.

Materials and Methods

Ethical Issues

In the present study, ethical issues are based on the permission of the Ethical Committee of the College of Medicine at the University of Babylon and the Ethical Committee of Babylon General Directorate of Health. As well as, persons participating in the present study were signed an informed consent.

Patients and Control

The sample size was determined according to the sample size equation. Fifty patients males with BPH clinically diagnosis by urologist attended to Al-Hillah Teaching Hospital, with a mean age of 45-80 years, as well as fifty apparently healthy control with a mean age of 45-60 years in a case-control study were subjected to present study

Diagnosis of Patients

Diagnosis of patients with BPH was done according to the PSA levels that were estimated in all of BPH patients with prostatic carcinoma proved by PSA, digital rectal examined and biopsy were conducted [12]. The full history was recorded and a comprehensive questionnaire was filled including age, gender, residence, length, weight, occupational status, past history of BPH, smoking, habits and if the patient suffered from any chronic diseases such as diabetes mellitus and hypertension.

The Single-Nucleotide Polymorphism of-34 T>C CYP17 Gene

DNA was extractedfrom blood using (Geneaid ® UK) kit.The CYP17 gene was detected by usingpolymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique in both BPH patients and control group, using the forward primer (5' CCA TTC GCA CTC TGG AGT CAT -3'), and reverse primer (5'-GAC AGG AGG CTC TTG GGG TA -3') to amplify 421 bp fragment. PCR optimization was done as a first step by using a gradient temperature ranging from 52 ºC to 54ºC. It is important to determine the optimum annealing temperature.

After the determination of optimum annealing temperature (54 ºC), the PCR reaction mixture was done by mixed 12.5 µL of master mix, 2.5 µLforward primer, 2.5 µLreverse primer, 5 µL templet DNA and 2.5 µL of nuclease-free water in a final volume of 25 µL. Amplification reactions were carried out by using thermocycler (Bioneer/ Korea) apparatus. After determination of the optimum annealing temperature, the following program was set in the thermocycler to amplify the target DNA fragments as shown in Table 1.

Table 1: Amplification conditions of CYP17 genotyping

Stage	Temp ºC	Time	Function	Cycles
1	94	30min.	Initial denaturation	
2	94	15-30sec.*	DNA denaturation	30**
	45-68	15-60 sec	Primer annealing	
	68	1 min/kb.	Template elongation	
3	68	5 min	Final elongation	
4	4-10	-	Incubation	Hold

* This degree from its reference with modification from 15 sec to 30 sec.
** the number of cycle was modified from 30 to 34 cycles.

The product of PCR was analyzed by electrophoresis on agarose gels electrophoresis.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism [13]

Polymerase chain response limitation part length polymorphism (PCR-RFLP) is utilized for genotyping relying upon restriction enzyme confinement endonuclease cleavage. The PCR item was processed with the confinement compound MspA1I, for codon 10 Gpx1: T→C (Leu→Pro) in exon 8 and product 421bp. RFLP was done in a 15 µL total reaction containing 10 U of MspA1I (Fermentase), 1x restriction enzyme buffer (10 mM Tris-HCl (pH 7.5 at 37 ºC), 10 Mm $MgCl_2$, 0.1 mg/ml BSA) and free DNase, RNase water at 65 ºC. PCR products were migrated by electrophoresis for 60 min at 70 V, 65 Am on a 2.5% agarose gel stained with Red Safe Stain MspA1I digestion resulted in two fragments of 291 and 130 bp for wild-type (pro/pro) T/T; remand one fragment without digested of 421 bp for variant homozygous(mutant) (Leu/Leu) C/C; and

three fragments of 421, 291 and 130 bp for heterozygous(pro/Leu) C/T, were visualized by UV light.

Restriction Enzyme Assay for Promoter Gene Polymorphism

For promoter gene polymorphism analysis, the PCR product was digested with the restriction enzyme MspA1I(restriction endonuclease).The optimization of digestion conditions was done by using:

- Different volume of enzyme (0.5 µL, and 1 µL).
- Working solution of enzyme was prepared by mixing 1 µL of stock enzyme with 4 µL enzyme buffer.
- Different volume of 10 X NE Buffer (4 µL and 5 µL).
- Different volume of PCR product (4 µL, 5 µL).
- Different incubation time (15-30min).
- The digestion conditions that gave best result were:

Final volume of reaction was 10 µL; consist of 4 µL from 10 X NE Buffer, 5 µL PCR product, 1 µL stock enzyme incubated at 65ºC for 30 min. The uncut fragment was 421 bp, whereas A1 (CC) homozygote digestion products were 421 bp, A2 (TT) homozygote digestion products were three bands (291and 130) bp. as well as A1\A2 (TC) Heterozygote digestion products were (421, 291 and 130) bp.

Results and Discussion

The CYP17 Polymorphisms

The -34 T > C CYP17 polymorphism was genotyped with a PCR method by confronting primers. Some samples were repeated twice to confirm PCR result. -34 T > C CYP17 genotyping was also performed by PCR-restriction fragment length polymorphism (RFLP-PCR). Genomic DNA was amplified by primers, forward, 5'- CCA TTC GCA CTC TGG AGT CAT -3'); and reverse primer (5'-GAC AGG AGG CTC TTG GGG TA -3'), followed by digestion with the restriction enzyme (MspA1I).

The CC (homozygous) genotype was indicated by two bands of 421bp, the TT genotype (homozygous) was indicated by three bands of 291/130bp, and the TC (heterozygous) genotype was indicated by the presence of three bands, that is, 421,291 and 130bp, as shown in Figure1.

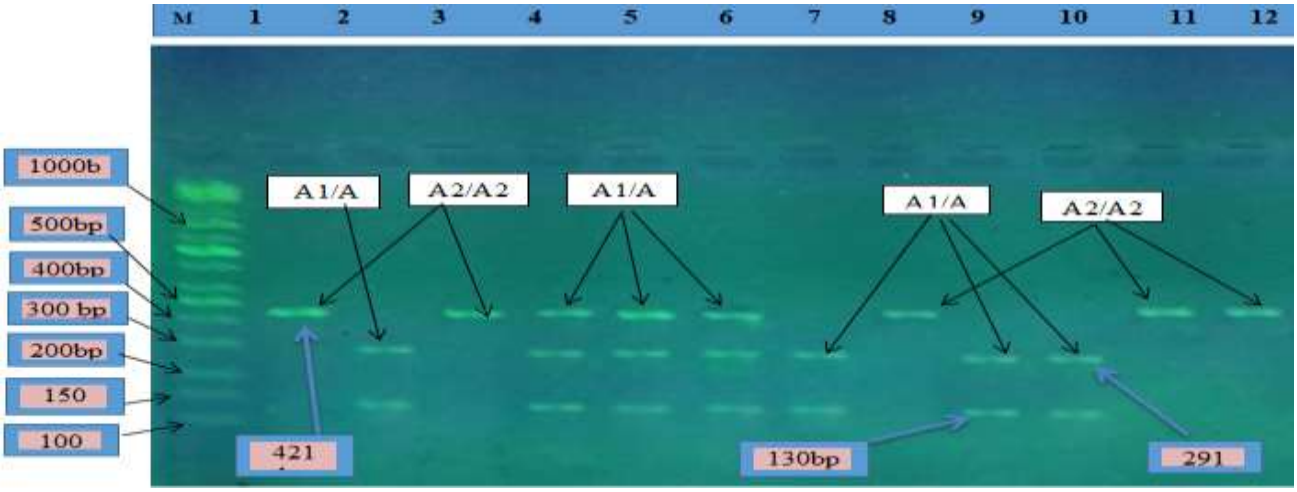

Figure 1Electrophoresis pattern of PCR product of CYP17 gene digested with *MspA1I*restriction enzyme (2.5% agarose gel). First 6 sample patientlane's (2,7) homozygous: CC genotype; Lane's(5) homozygous: TT genotype; Lane's (12,13,16) heterozygous TC genotype. Second 6 Sample Control Lane's (7,7,7) homozygous: CC genotype; Lane's(3, 9,17) homozygous: TT genotype; Lane's (0) heterozygous TC genotype. M: DNA molecular marker 100bp size by red stain stained bands in the gel

Table 2 summarizes positive results of the -34 T > C CYP17 allele of RFLP in samples study (patients and control). The result shows high significant (P<0.00048) changes when compared between patients and control groups.

Table 2: Genotype distribution of CYP17gene in healthy control and patients groups

	Control		Patients		OR (CI 95%)	P-value
Allele	No.	Proportion	No.	Proportion		
C	65	0.72	42	0.47	0.337 (0.181 -0.626)	0.00048
T	25	0.28	48	0.53	2.971(1.599-5.523)	

To determine odds ratios and 95% confidence intervals for BPH risk, various comparisons of genotypes, as well as C vs. T alleles, were analyzed. The odds ratio for comparison between benign prostatic hyperplasia with control groups for C allele subjects were 0.337 (95%, CI 0.181-0.626), while the T allele subjects were 2.971 (95%, CI1.599-5.523).

Genotypes Association

The genotypes association is shown in Table 3. The model codominant found to change significantly with (P=0.0001) when compared with healthy control. The model dominant found to change significantly with (P =0.0001) when compared with healthy control according to the percentage of T/C-T/Tis genotypes response and related to BPH risk. The recessive model found to change non-significantly with (P =0.44) when compared with healthy control. The model overdominant found to change significantly with (P =0.0001) when compared with healthy control according to the percentage of T/C is genotypes response and related with BPH, as shown in Table 3.

Table 3 The Genotypes Association of -34 T > CCYP17 Polymorphism in patients and Healthy Control

Model	Genotype	Control	Patients	OR (95% CI)	P-value
Codominant	C/C	31 (68.9%)	5 (11.1%)	1.00	<0.0001
	T/C	3 (6.7%)	32 (71.1%)	66.13 (14.55-300.63)	
	T/T	11 (24.4%)	8 (17.8%)	4.51 (1.21-16.75)	
Dominant	C/C	31 (68.9%)	5 (11.1%)	1.00	<0.0001
	T/C-T/T	14 (31.1%)	40 (88.9%)	17.71 (5.76-54.49)	
Recessive	C/C-T/C	34 (75.6%)	37 (82.2%)	1.00	0.44
	T/T	11 (24.4%)	8 (17.8%)	0.67 (0.24-1.86)	
Overdominant	C/C-T/T	42 (93.3%)	13 (28.9%)	1.00	<0.0001
	T/C	3 (6.7%)	32 (71.1%)	34.46 (9.05-131.22)	

Results of present study found evidence for genetic association conferred by -34 T > C CYP17 gene promoter polymorphisms with respect to susceptibility to BPH and, there were 53% with T allele promoter polymorphism, 47% with C in patients group, and 28% with T allele promoter polymorphism, 72% with C in the control group. The present study is agreed with Stanford JL, et al. [14] that found an association between polymorphism of -34 T > C CYP17 gene with BPH. They according to their results suggest that -34 T > C CYP17 gene polymorphisms can associate with BPH susceptibility and may play a critical role in the pathogenesis of BPH [15,16]. The result of the present study is also agreed to a study conducted in China by Lin-Lin Zhang et al. [17].

Conclusion

The -34 T > C CYP17 gene polymorphism could have an important role in the susceptibility and pathogenesis of BPH.

Conflict of Interest

There is no conflict of interest in the present study.

References

1. Yeboah Ed, Ann W (2016) HsingBenign Prostatic Hyperplasia And Prostate Cancer In Africans And Africans In The Diaspora.J West Afr Coll Surg., 6(4): X-Xviii.
2. Homma Y, Gotoh M, Yokoyama O, Masumori N, Kawauchi A, Yamanishi T, et al (2011) Outline of JUA clinical guidelines for benign prostatic hyperplasia. Int. J. Urol., 18(11): 741-56.
3. Sarma A V, Wei JT (2012) Benign prostatic hyperplasia and lower urinary tract symptoms. N. Engl. J. Med., 367(3):248-57.
4. Lee HW, Kim SA, Nam JW, et al (2014) The study about physical activityfor subjects with prevention of benign prostate hyperplasia. Int Neurourol J., 18(3):155-162.
5. Parsons JK (2010) Benign prostatic hyperplasia and male lower urinary tract symptoms: epidemiology and risk factors. Curr Bladdr Dysfunct Rep., 5(4):212-218.
6. Chapple CR (2004) Pharmacological therapy of benign prostatic hyperplasia/lower urinary tract symptoms: an overview for the practising clinician. BJU Int., 94(5):738-744.
7. Lam JS, Cooper KL, Kaplan SA (2004) Changing aspects in the evaluation and treatment of patients with benign prostatic hyperplasia. Med Clin., 88(2):281-308.
8. McConnell JD (1991) The pathophysiology of benign prostatic hyperplasia. J. Androl. 12(6):356-63.
9. Habuchi T, Liqing Z, Suzuki T, Sasaki R,

Tsuchiya N, Tachiki H, et al (2000) Increased risk of prostate cancer and benign prostatic hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. Cancer Res, 60(20):5710-3.

10. Alta'ee A H, Ewadh M J, Kamil Z H (2016) Role of Some Trace Elements in Breast Cancer Receiving Chemotherapy, Int. J. Phar. Tech. Res, 9 (5): 381-386.
11. Daviglus ML, Dyer AR, Persky V, Chavez N, Drum M, Goldberg J, et al (1996) Dietary beta-carotene, vitamin C, and risk of prostate cancer: results from the Western Electric Study. Epidemiology, 7(5) :472–7.
12. Ananthan V, Sudha Presanna B, Pragna B, Dolia B, Ramadesikan VK, Sumathy S, Shanmuga Priya C (2016) Association of CYP17 Gene Polymorphism with Benign Prostatic Hyperplasia, Sch. J. App. Med. SCI., 4 (7E): 2630-2635.
13. Alanzy AK, Alta'ee AH, Alrubiae SJ (2018) Serum Tumor Necrosis Factor Alpha and Gene Polymorphisms in Rheumatoid Arthritis Patients in Babylon Province, Iraq, Journal of Global Pharma Technology, 10 (3): 387-395
14. Habuchi T, Liqing Z, Suzuki T, Sasaki R, Tsuchiya N, Tachiki H, Shimoda N, Satoh S, Sato K, Kakehi Y, Kamoto T (2000) Increased risk of prostate cancer and benign prostatic hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. Cancer research, 60(20):5710-3.
15. Sharp L, Cardy AH, Cotton SC, Little J (2004) CYP17 gene polymorphisms: prevalence and associations with hormone levels and related factors. A HuGE Review. Am. J. Epidemiol., 160: 729-740.
16. Kumar V, Yadav CS, Singh S, Goel S, Ahmed RS, Gupta S, Grover RK, Banerjee BD (2010) CYP 1A1 polymorphism and organochlorine pesticides levels in the etiology of prostate cancer.Chemosphere, 81(4):464-468.
17. Lin-Lin Zhang1, Yu Song2, Ling-Ling He2, Guo-Qiang Chen3, Ji-Cheng Fu3, Lei Liu2, Bao Xi1, Lei Wang2 (2017) Associations of SRD5A2/CYP17/CYP19/VDRgene polymorphisms with the development andclinical progression of benign prostatic hyperplasia: case-control study in northern Chinese population Int. J. Clin. Exp. Pathol.,10(8):8660-867.

