

Original Research Article

Association of CYP17 Gene Polymorphism with Benign Prostatic Hyperplasia**Dr. V. Ananthan, Dr. B. Sudha Presanna, Dr. Pragna. B. Dolia, Dr. V.K. Ramadesikan, Dr. S. Sumathy,
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Abstract: The CYP17 gene encodes for the Cytochrome P450c17 α enzymes 17 α hydroxylase, 17-20 lyase in testosterone synthesis. There is a polymorphism (T to C substitution) in the 5' untranslated region of CYP17 gene, providing an increased rate of transcription of CYP17 mRNA, which in turn increases enzyme cytochrome P-450c17 α synthesis, increases androgen production and increase cell division in the prostate, thereby increases the risk of BPH. Genotype analysis was done on 50 patients with proven BPH and 50 healthy controls by polymerase chain reaction followed by restriction digestion. Men with A2/A2 CYP17 genotype had an increased risk of BPH with an odds ratio (OR), of 2.88; 95% confidence interval (CI) = [1.1-3.8] compared with those with the A1/A1 genotype. The trend of an increasing risk of BPH with an increasing number of A2 allele was statistically significant. The A2 allele of the CYP17 polymorphism is associated with an increased risk of BPH.**Keywords:** CYP17 gene polymorphism, Benign Prostatic Hyperplasia, restriction digestion

INTRODUCTION:

Benign prostatic hyperplasia is the most common benign tumor in men, and its incidence is age-related. BPH refers to the stromal and epithelial proliferation in the prostate gland that may eventually result in voiding symptoms. Benign prostatic hyperplasia is truly a hyperplastic process, resulting from an increase in cell numbers. The prevalence of histological benign prostatic hyperplasia studies rises from approximately 20% in men aged 41–50 years, to 50% in men aged 51–60, 70% of men in their seventies and to over 90% in men over 80 years of age and in nearly all men in their nineties [1]. Risk factors for the development of benign prostatic hyperplasia are poorly understood. Some studies have suggested a genetic predisposition and some have noted racial differences. Approximately 50% of men under age 60 years who undergo surgery for benign prostatic hyperplasia may have a heritable form of the disease. The etiology is not completely understood, but the disorder seems to be multifactorial and under endocrine control. Laboratory and clinical studies have identified two factors necessary for the development of benign prostatic hyperplasia: dihydrotestosterone (DHT) and aging. Dihydrotestosterone is very essential for prostate growth. The serum testosterone levels decrease as a result of aging mainly due to decreased stimulation of

leydig cells and increased conversion of testosterone in the peripheral tissues to estrogens which induce androgen receptor. The role of androgen is to initiate stromal hyperplasia or benign Prostatic hyperplasia via the autocrine or paracrine mediators. Animal studies have demonstrated that the aging prostate becomes more sensitive to androgens. Further investigations have demonstrated a positive correlation between levels of free testosterone and estrogen and the volume of the gland. The latter may suggest that the association between aging and benign prostatic hyperplasia might reflect increasing estrogen levels of aging, resulting in induction of the androgen receptor and thus sensitizing the prostate to free testosterone. Steroid hormones are suspected to play a role in the growth of prostate neoplasia and expression of genes regulating steroid hormone levels may thereby affect the disease risk [2, 3]. There is an evidence to support the hypothesis of hormonal etiology of BPH involving androgen action.^{2, 3} Androgen is required for differentiation and growth of the prostate in utero and at puberty [12]. Testosterone, the most abundant circulating androgen is synthesized from cholesterol by a series of enzymatic reactions involving the enzyme cytochrome p450c17 α . The Cytochrome P-450c17 α (CYP17) gene, is located on chromosome 10q24.3, spans 6569 bp and is divided into eight exons, encodes the enzyme cytochrome P-450c17

α , which functions at key branch points in steroid hormone biosynthesis in the adrenal gland, ovary, and gonads [4, 5]. Specifically, cytochrome P-450c17 α mediates both steroid 17 α -hydroxylase activity, which converts pregnenolone to dehydroepiandrosterone, and 17,20-lyase activity, which generates androstenedione from progesterone, precursors of testosterone and estrogen. These androgens may then be converted to estrone, testosterone, and estradiol. Testosterone is converted to dihydrotestosterone (DHT) in the prostate by the enzyme 5 α -reductase. DHT binds to AR (androgen receptor), and the DHT-AR complex transactivates a number of genes with AR responsive elements. These events ultimately result in cell division in the prostate [6, 7].

There are numerous mutations in the CYP17 gene, the majority of which are extremely rare [8, 9]. Three common polymorphisms have been described,¹⁰⁻¹² but only one, a single nucleotide polymorphism (SNP) in the 5'-untranslated promoter region of CYP17 (5'-UTR) is found to be associated with prostate neoplasia. The 5'-untranslated promoter region of CYP17 contains a single base pair T to C polymorphism that may create a new Sp-1 site (CCACC box) at 34 bp upstream from the initiation of translation and downstream from the putative transcription start site, therefore providing an additional promoter activity with an increased rate of transcription of CYP17 mRNA, which in turn increases enzyme cytochrome P-450c17 α synthesis(activity), increases androgen production and increase cell division in the prostate, thereby increases the risk of BPH. Pair designate CC, heterozygosity as "A1A2" (rather than CT), and homozygosity for the variant allele as "A2A2" (rather than TT).

MATERIALS AND METHODS:

STUDY POPULATION:

CASES:

The study sample comprised 50 Benign Prostatic Hyperplasia patients with mean age 54.31 + 8.69. All of the BPH patients had various degrees of lower urinary tract symptoms and showed an apparent prostatic enlargement by digital rectal examination and ultrasonogram. The PSA levels were measured in all of the BPH patients, and men with elevated PSA levels (≥ 4.0 ng/ml) were proved not to have prostate cancer by transrectal biopsies.

CONTROL SUBJECTS:

The male control group consisted 50 volunteers > 60 years old who were selected mainly from among the patients who attended outpatient departments of Geriatrics with non-urological diseases and showed no signs of prostate cancer and no Prostatic enlargement by digital rectal examination. They all were tested for serum PSA levels and men with abnormal PSA levels were excluded from the normal controls.

METHODS:

Height and weight were recorded and blood samples were collected by Venipuncture after overnight fasting in two test tubes. One was plain tube and the other anticoagulated with Disodium EDTA. Plain tube was centrifuged and serum was aliquoted for PSA level estimation. Disodium EDTA tube was centrifuged at 2000 rpm for twenty minutes to get the buffy coat for DNA extraction.

CYP17 Gene Polymorphism Screening:

DNA was extracted from buffy coat by high salt method¹³ and was used to amplify the 421bp target region in the CYP17 gene by PCR using forward 5'-CCA TTC GCA CTC TGG AGT CAT - 3' and reverse 5'- GAC AGG AGG CTC TTG GGG TA- 3' primers. Genomic DNA (1 μ g) was amplified in 25 μ l (PCR master mix 12.5 μ L, Forward primer 0.8 μ L, Reverse primer 0.8 μ L, DNA 2 .0 μ L, Distilled water 8.9 μ L) reaction mixture containing 0.3 μ mol/L of each primer and red dye master mix (Bangalore Genei) containing 100 μ mol/L of each dNTP, 2.5 μ L of 10x reaction buffer and 0.6 unit of Taq DNA polymerase. After the DNA was denatured for 5 minutes at 94 $^{\circ}$ C, the reaction mixture was subjected to 30 cycles of denaturation for one minute at 94 $^{\circ}$ C, 1 minute of annealing at 50 $^{\circ}$ C and 1 minute of extension at 72 $^{\circ}$ C. Final extension was carried over at 72 $^{\circ}$ C for 6 minutes. CYP17 polymorphism was detected by digestion of the PCR amplified product with 10 units of MspAII restriction enzyme (New England Biolabs) for overnight followed by size fractionation in 3% Agarose Gel Electrophoresis. A1 allele does not have the restriction site hence will yield a 421bp fragment, A2 allele has the restriction site, hence gets cleaved to give 130bp and 291bp fragment. Analysis was done using a low molecular weight DNA ladder (100 bp).

PSA level was measured by ELISA methods with an open system automated ELISA analyzer (Triturus analyzer).

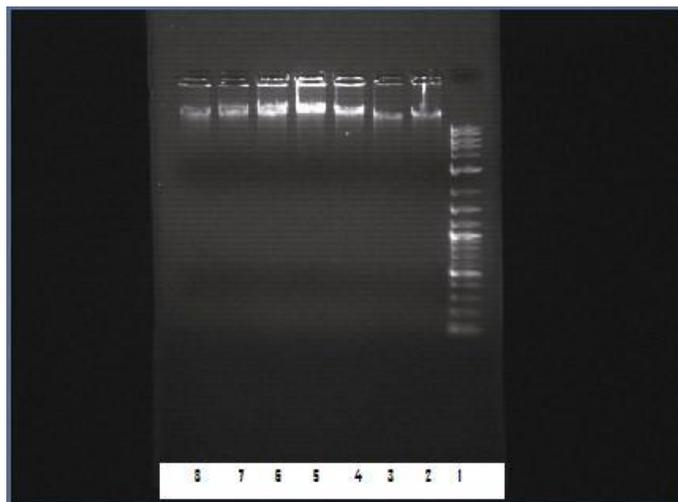


Fig 1: Extracted DNA (lane 2 to 8) was tested on 1% agarose gel using 1kb ladder (lane 1) Ladder shows 10000, 8000, 7000,6000, 5000, 4000, 3000, 2000,1000 bp fragments

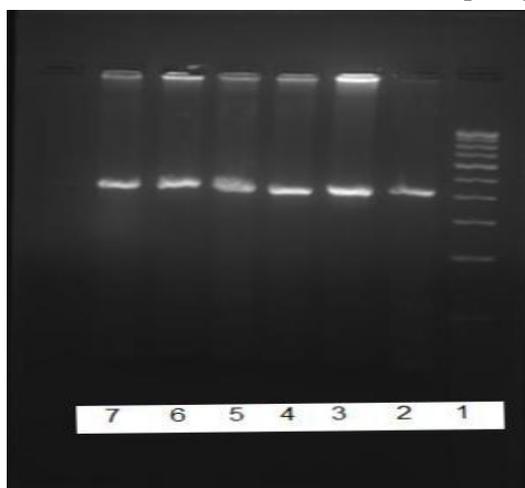


Fig 2: the 421bp CYP17 gene PCR product (lane 2 to 7) on 2 % agarose gel. Lane 1 shows 100bp DNA ladder – marker fragments include 1000, 900, 800, 700, 600, 500, 400, 300, 200,100 bp.

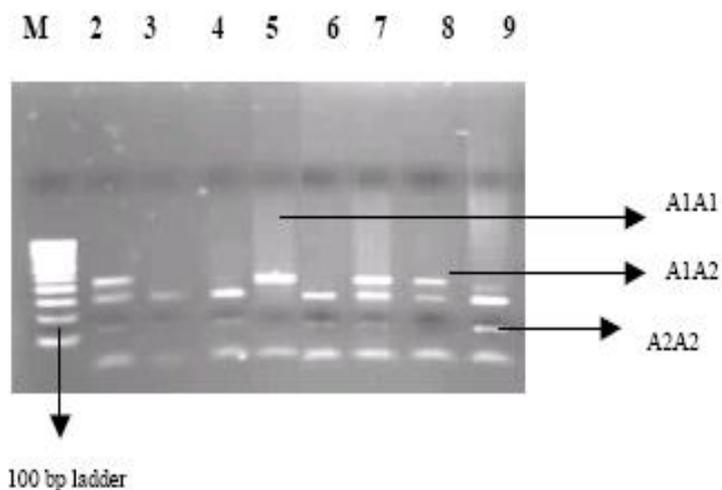


Fig 3: CYP 17 genotypes after MspA1 digestion, showing the three fragment sizes (421, 291, and 130 bp). Lane 1, Molecular weight marker (100-bp ladder); lanes 2, 7, and 8, heterozygote A1/A2 (TC); lane 5, homozygote A1/A1(TT); lanes 3, 4,6, and 9, homozygote A2/A2(CC).

Statistical Analysis:

Allele frequencies were calculated by allele counting. Age and BMI were compared between control subjects and patients by students "t" test. Genotype frequency distribution between cases and controls were compared with a χ^2 test for 2*2 contingency table. Logistic regression analysis was performed to evaluate the interaction between human CYP17 gene and other variables in relation to the prevalence of BPH. Independent variables included in the analysis were age, smoking, Alcoholism, Hypertension, Diabetes. The analysis was executed by SAS Statistical program Version 6.10 for Macintosh. Relationship between the number of BPH and the genotype was assessed by Spearman's Rank Correlation analysis.

RESULTS:

Table 1 shows Age, BMI, PSA levels and conventional risk factor distribution among benign

prostatic hyperplasia and control subjects. Since all the confounding factors were matched there were no significant differences between cases and controls.

Table 2 & 3 shows Genotype distribution and Allele frequencies of human CYP17 gene in patients with benign prostatic hyperplasia and control subjects. The Allele frequencies were A1/A1 = 30, A2/A2 =36 and A1/A2 = 34. This was found to be in Hardy Weinberg equilibrium. χ^2 value is 31.92, p value is 0.031. A2 genotype was more frequent among cases (44%) when compared to controls (24%). In contrast A1 was more common among controls (40%) when compared to cases (20%). There was a significant difference in the distribution of A2 genotype also between cases (80%) and controls (60%). P value is 0.02. In short A2+ genotype is more common among cases (80%) when compared to controls (60%). P value is 0.02.

Table 1: Characteristics of Patients with Benign Prostatic hyperplasia and of Control Subjects

Variables	Case	Control	p value
Age	62.20± 9.66	69.20 ± 5.40	0.68 –NS
DM	18 (34%)	19 (38.0%)	0.51 –NS
HT	23 (44%)	24(48%)	0.47 –NS
DM+HT	15 (32%)	17 (34%)	0.53 – NS
SMK	28 (56%)	25(50%)	0.49 –NS
ALC	26 (54%)	30 (60%)	0.36 –NS
BMI	27.42±3.82	25.42±3.48	0.09 –NS

Table 2: Genotype distribution of human CYP17 gene with Benign Prostatic Hyperplasia

Genotype	Control	Case	P value
A1/A1	20 (40%)	10(20%)	Chi sq = 31.92 P = .031 – S
A1/A2	18 (36%)	18 (36%)	
A2/A2	12(24%)	22 (44%)	

Table 3: Allele frequencies of human CYP17 gene with Benign Prostatic Hyperplasia

Genotype	Control	Case	P value
A2+*	30(60%)	40(80%)	Chi sq = 28.36 P = .02 – S
A2-*	20 (40%)	10 (20%)	

A2+* → A2/A2 + A1/A2

A2-* → A1/A1

DISCUSSION:

We evaluated the association between BPH and polymorphisms in the CYP17 gene which is involved in the biosynthesis and metabolism of testosterone. Because testosterone and DHT are potent prostate mitogens, elevated levels in prostate tissue are hypothesized to play a role in unregulated prostate growth and tumorigenesis and have been shown to be associated with increased risk of prostate growth and enlargement. We found some evidence that the putative high activity allele (A2) of the CYP17 gene, which would be predicted to increase levels of testosterone, may be associated with BPH (OR = 1.7, 95% CI = 1.0–3.0; P = 0.04).

Although conflicting results have been documented, the presence of the CYP17 A1 allele has been described to be an independent risk factor for prostate cancer and BPH. However, the present results indicated that the presence of the A2 allele significantly increases the risk of prostate cancer and BPH. Because cytochrome P450c17 encoded by CYP17 has both 17 -hydroxylase and 17, 20-lyase activities, CYP17 is involved in the production of both androgens and estrogens. It has been well accepted that most prostate cancers are androgen-dependent and that an androgen defect prevents normal prostate growth, whereas most breast cancers are estrogen-dependent and estrogens

have promoting effects on breast carcinogenesis. Consequently, the present results, together with those of the previous documents, suggest that the A2 allele has a more androgenic effect on men and estrogenic effect on women.

On the other hand, three previous studies reported conflicting results on the CYP17 genotype in prostate cancer patients. One from the United States indicated an increased risk of prostate cancer in the presence of the A2 allele (OR, 1.7; 95% CI = 1.0–3.00)[18], whereas another from Sweden claimed that men with the A1/A1 genotype had an increased risk (OR, 1.61; 95% CI = 1.02–2.53)[19]. More recently, Gsur *et al.*; reported an increased risk in men with the A2/A2 genotype in a small cohort of prostate cancer patients in Austria[20]. The conclusion in the United States study seems to remain unchanged even when the analysis is restricted to a Caucasian population. Although the exact reason for these contradictory results remains unclear, the identical CYP genotype may play either a protective or a promoting role in prostate carcinogenesis given different environmental and/or genetic backgrounds. In support of this view, studies showed that women with an A2/A2 genotype had higher levels of estradiol and estrone and that the A2 allele was associated with significantly higher levels of estradiol, whereas the A2 allele was associated with phenotypic modification of a familial form of polycystic ovaries whose sex steroid hormone balance has been shown to be more androgen-dominant than normal. These documents suggest that even women with the identical CYP17 genotype have much different phenotypes as far as hormone-dependent diseases are concerned. Because of the multiple enzymatic processes required for steroid hormone synthesis, the specific step which leads to enzyme hyperactivity may result in either a hyperestrogenic or a hyperandrogenic hormonal status according to the difference in activities of the other enzymatic processes which follow.

Our results indicated that the CYP17 genotype is associated with the development of BPH. This connection is in line with the observation that a subset of BPH has a genetic transmission [14]. It has been reported that the volume of BPH is positively correlated with serum testosterone, estradiol, and estrion levels [15], therefore indicating imbalance of the androgen and estrogen resulting in development of BPH. A distinct sex-steroid hormone environment caused by the CYP17 genotype will presumably contribute to the development of BPH as well as prostate cancer. On the other hand, BPH and prostatic cancer arise from a different part of the prostate gland, and BPH itself presumably does not substantially increase the risk of clinically significant prostate cancer [16]. These findings suggest that the CYP genotype is involved in distinct pathways of cellular growth of the prostate gland.

CONCLUSION:

The present study indicated that CYP17 gene polymorphism may be significantly associated with a risk of BPH.

REFERENCES:

1. Cunha GR, Chung LW, Shannon JM, Taguchi O, Fujii H; Hormone-induced morphogenesis and growth: Role of mesenchymal-epithelial interactions. *Recent Prog Horm Res* 1982; 39:559-598.
2. Gann P.H, Hennekens C.H, Ma J, Longcope C, Stampfer M.J; Prospective study of sex hormone levels and risk of prostate cancer [see comments]. *J. Natl Cancer Inst.*, 1996; 88: 1118–1126.
3. Wilding G; The importance of steroid hormones in prostate cancer. *Cancer Surv* 1992; 14: 113– 130.
4. Ilic M, Vlajinac H, Marinkovic J; Case-control study of risk factors for prostate cancer. *Br. J. Cancer*, 1996; 74: 1682–1686.
5. Daviglus M.L, Dyer A.R, Persky V, Chavez N, Drum M, Goldberg J, *et al.*; Dietary beta-carotene, vitamin C, and risk of prostate cancer: results from the Western Electric Study, *Epidemiology*, 1996; 7: 472–477.
6. Galbraith S.M, Duchesne G.M; Androgens and prostate cancer: biology, pathology and hormonal therapy. *Eur. J. Cancer*, 1997; 33: 545–554.
7. Brentano ST, Picado-Leonard J, Mellon SH, Moore C.C, Miller W.L; Tissue- specific, cyclic adenosine 3',5'-monophosphate-induced, and phorbol ester-repressed transcription from the human P450c17 promoter in mouse cells. *Mol Endocrinol* 1990; 4(12):1972–9.
8. Lumey L.H; Prostate cancer and smoking: a review of case-control and cohort studies [published erratum appears in *Prostate*, 1996; 29: 249–260.
9. Van der Gulden JW, Kolk J.J, Verbeek A.L; Prostate cancer and work environment [see comments]. *J. Occup. Med.*, 1992; 34: 402–409.
10. Whittemore A.S; Trends in prostate cancer incidence and mortality. In Doll,R., Fraumeni,J.F. and Muir,C.S. (eds), *Cancer Surveys*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994.
11. Parkin D.M, Pisani P, and Ferlay J; Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer*, 1993; 54: 594–606.
12. Neuhausen S.L, Skolnick M.H, Cannon-Albright L; Familial prostate cancer studies in Utah. *Br. J. Urol.*, 1990; 79 (Suppl. 1): 15–20.
13. Steinberg, G.D, Carter B.S, Beaty T.H., Childs B, Walsh P.C; Family history and the risk of prostate cancer. *Prostate*, 1997; 17: 337–347.
14. Partin A.W, Coffey D.S; The molecular biology, endocrinology, and physiology of the prostate and seminal vesicles. In: P. C. Walsh, A. B. Retik, E. D. Vaughan, Jr., and A. J. Wein (eds.), *Canbell's*

- Urology , 3rd edition, Philadelphia: W. B. Saunders Co., 1998; 1381–1428.
15. Makridakis N, Reichardt J, Pike M, Stanczyk F, Yu M, Kolonel L *et al.*; Genetic control of human androgen metabolism by the CYP17 and SRD5A2 Genes. *Am. J. Hum. Genet*, 1997; 61: A257.
 16. Spurdle A. B, Hopper J.L, Dite G.S, Chen X, Cui J, McCredie M.R.E, *et al.*; CYP17 promoter polymorphism and breast cancer in Australian women under age forty years. *J. Natl. Cancer Inst.*, 2000; 92: 1674-1681.
 17. Feigelson H.S, Ross R.K, Yu M.C, Coetzee G.A, Reichardt J. K.V, Henderson B.E; Sex steroid hormones and genetic susceptibility to breast and prostate cancer. *Drug Metab. Rev.*, 1998; 30: 421-434.
 18. Stanford JL, Noonan EA, Iwasaki L, Kolb S, Chadwick RB, Feng Z, Ostrander EA; A polymorphism in the CYP17 gene and risk of prostate cancer. *Cancer Epidemiology Biomarkers & Prevention*. 2002;11(3):243-7.
 19. Habuchi T, Liqing Z, Suzuki T, Sasaki R, Tsuchiya N, Tachiki H, Shimoda N, Satoh S, Sato K, Kakehi Y, Kamoto T; Increased risk of prostate cancer and benign prostatic hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. *Cancer research*. 2000;60(20):5710-3.
 20. Gsur A, Bernhofer G, Hinteregger S, Haidinger G, Schatzl G, Madersbacher S, Marberger M, Vutuc C, Micksche M; A polymorphism in the CYP17 gene is associated with prostate cancer risk. *International journal of cancer*. 2000;87(3):434-7.