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Original Research Article

Association of CYP17 Gene Polymorphism with Carcinoma of Prostate

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Abstract: The CYP17 gene encodes for the Cytochrome P450c17α enzymes 17α 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-450c17α synthesis, increases androgen production and increase cell division in the prostate, thereby increases the risk of carcinoma of prostate. Genotype analysis was done on 50 patients with proven carcinoma of prostate and 50 healthy controls by polymerase chain reaction followed by restriction digestion. Men with A2/A2 CYP17 genotype had an increased risk of carcinoma of prostate with an odds ratio (OR), of 2.13; 95% confidence interval (CI) =1.33-3.51] compared with those with the A1/A1 genotype. The trend of an increasing risk of carcinoma of prostate with an increasing number of A2 allele was statistically significant. The A2 allele of the CYP17 polymorphism is associated with an increased risk of carcinoma of prostate.
Keywords: CYP17 gene polymorphism, carcinoma of prostate, restriction digestion.

INTRODUCTION

Prostate cancer is the most common malignancy and the third most common cause of cancer mortality among men [1]. Studies of risk factors such as occupation, diet, smoking, alcohol and sexual activity are still inconclusive [2-5]. However, age, ethnicity and family history clearly affect the risk of prostate cancer [6-9]. Steroid hormones are suspected to play a role in the growth of prostate cancer, and expression of genes regulating hormone levels may thereby affect disease risk [10, 11]. There is evidence to support the hypothesis of hormonal etiology of prostate cancer involving androgen action [10, 11]. 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 p450c17a.

The Cytochrome P-450c17α (CYP17) gene, is located on chromosome 10q24.3, spans 6569 bp and is divided into eight exons, located on chromosome 10q24.3, encodes the enzyme cytochrome P-450c17α, which functions at key branch points in steroid hormone biosynthesis in the adrenal gland, ovary, and gonads 2,3. Specifically, cytochrome P-450c17α mediates both steroid 17α-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 5α-dihydrotestosterone (DHT) in the prostate by the enzyme 5 α -reductase. DHT binds to AR (androgen receptor gene), and the DHT–AR complex transactivates a number of genes with AR responsive elements. These events ultimately result in cell division in the prostate.

There are numerous mutations in the CYP17 gene, the majority of which are extremely rare [4,5]. Three common polymorphisms have been described [6-8], 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-bp 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 [6], which in turn increases enzyme cytochrome P-450c17α synthesis(activity), increases androgen production and increase cell division in the prostate, thereby increases the risk of Prostate cancer.

The variant creates a recognition site for the MspAI restriction enzyme. We will refer to the common allele as A1 and the variant allele, –34T→C, as A2. Thus, we will refer to homozygosity for the common allele as "A1A1" rather than use the base-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 unrelated Prostatic cancer patients of mean age 53.34 + 9.84 years. All of the prostate cancer patients were diagnosed histologically with specimens obtained from transrectal needle biopsy or transurethral resection of the prostate for voiding symptoms. The PSA levels were measured in all of the prostate cancer, and men with elevated PSA levels (≥4.0 ng/ml) were proved to have prostate cancer by transrectal biopsies. Serum PSA was measured using the Tandem-R assay in most cases.

Pathological grading of the prostate cancer was determined according to the General Rule for Clinical and Pathological Studies on Prostate Cancer which is based on the WHO criteria and the Gleason pattern. Well, moderately, and poorly differentiated carcinoma generally corresponds to Gleason patterns 1–2, 3– 4, and 5, respectively . The clinical or pathological stage was determined by review of the medical records and classified using the Tumor-Node-Metastasis system. Prostate cancer was classified into the localized group consisting of T1-4N0M0 (stage A, B, or C by the Whitmore-Jewett system) tumors and the metastatic group consisting of T1–4N1M0–1 or T1–4N0–1M1 (stage D by the Whitmore-Jewett system) tumors.

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 method[13] 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 (1µg) was amplified in 25µl (PCR master mix 12.5 µL, Forward primer 0.8 µL, Reverse primer 0.8 µL, DNA 2 .0µL, Distilled water 8.9 µL) reaction mixture containing 0.3µmol/L of each primer and red dye master mix (Bangalore Genei) containing 100µmol/L of each dNTP, 2.5µL of 10x reaction buffer and 0.6 unit of Taq DNA polymerase. After the DNA was denatured for 5 minutes at 94ºC, the reaction mixture was subjected to 30 cycles of denaturation for one minute at 94ºC, 1 minute of annealing at 50 ºC and 1 minute of extension at 72 ºC. Final extension was carried over at 72 ºC for 6 minutes. CYP17 polymorphism was detected by digestion of the PCR amplified product with 10 units of MspA1I 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).

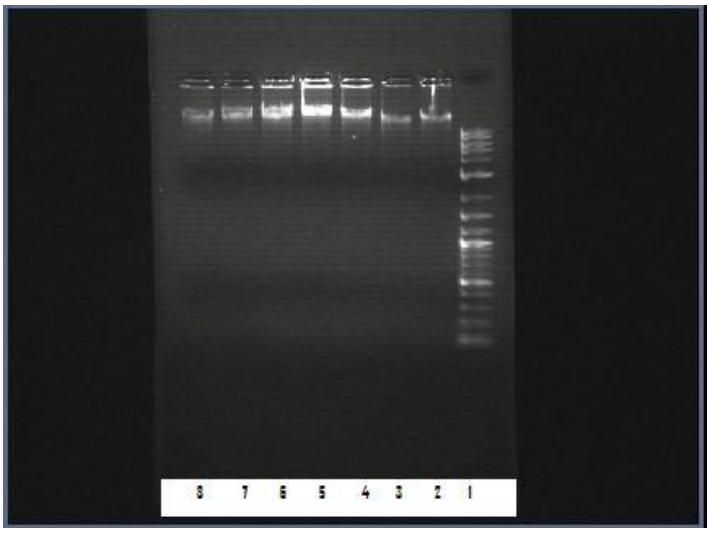

Fig-1: Extracted DNA (lane 2 to 8) was tested on 1% agarose gel using 1kb ladder (lane 1) Ladder shows 10000, 8000, 7000,6000, 5000, 4000, 3000, 2000,1000 bp fragments

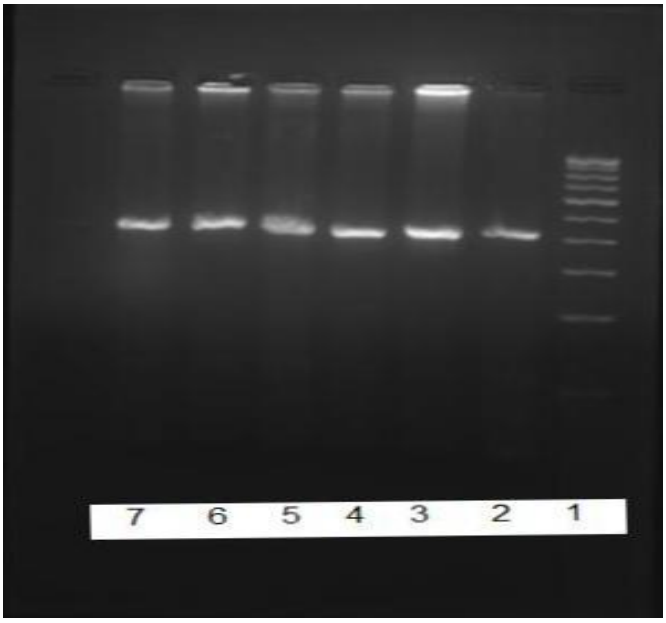

Fig-2: shows the 421bp CYP17 gene PCR product (lane 2 to 7) on 2 % agarose gel. Lane 1 shows 100bp DNA ladder – marker fragments include 1000, 900, 800, 700, 600, 500, 400, 300, 200,100 bp.

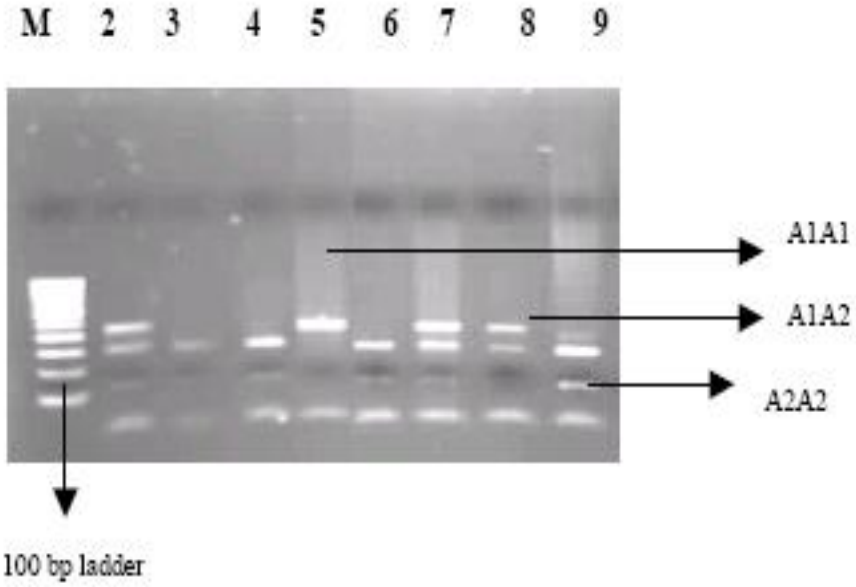

Fig-3: Shows *CYP 17* genotypes after *Msp*A1 digestion, showing the three fragment sizes (421, 291, and 130 bp). Lane 1, Molecular weight marker (100-bp ladder); lanes 2, 7, and 8, heterozygote A1/A2 (TC); lane 5, homozygote A1/A1(TT); lanes 3, 4,6, and 9, homozygote A2/A2(CC).

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 prostate cancer. 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 prostate cancer 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 prostate cancer 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. $\varkappa^2$ value is 32, 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 0.03. 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 Prostate Cancer and of Control Subjects.

Variables	Case	Control	P value
Age	59.34 ± 9.84	60.16 ± 10.11	0.86 –NS
DM	17 (34%)	19 (38.0%)	0.71 –NS
HT	22 (44%)	24(48%)	0.46 –NS
DM+HT	16 (32%)	17 (34%)	0.62- NS
SMK	28 (56%)	25(50%)	0.46 –NS
ALC	27 (54%)	30 (60%)	0.33 –NS
BMI	26.51 ±3.39	25.89 ± 3.29	0.08 –NS

Table-2: Genotype distribution of human CYP17 gene with Prostate Cancer.

Genotype	Control	Case	P value
A1/A1	20 (40%)	7 (14%)	Chi sq = 32
A1/A2	18 (36%)	19 (38%)	P = 0.031 – S
A2/A2	12(24%)	24 (48%)	

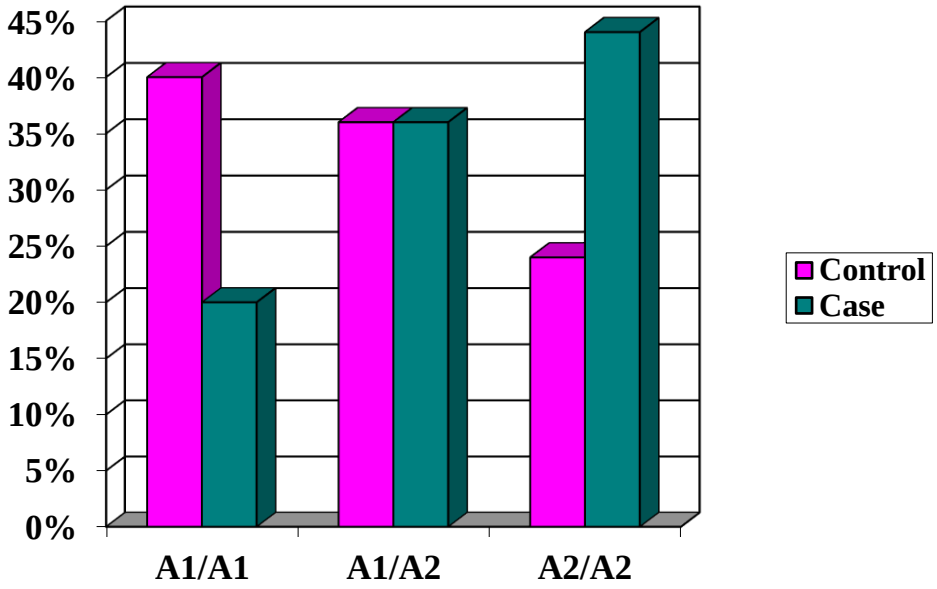

Fig-1: Genotype distribution of human CYP17 gene with Prostate Cancer

Table-3: Allele frequencies of human CYP17 gene with Prostate Cancer.

Genotype	Control	Case	P value
A2+*	30(60%)	43(86%)	Chi sq = 31.98
A2-*	20 (40%)	7 (14%)	P = .002 – S

A2+* → A2/A2 + A1/A2
A2-* → A1/A1

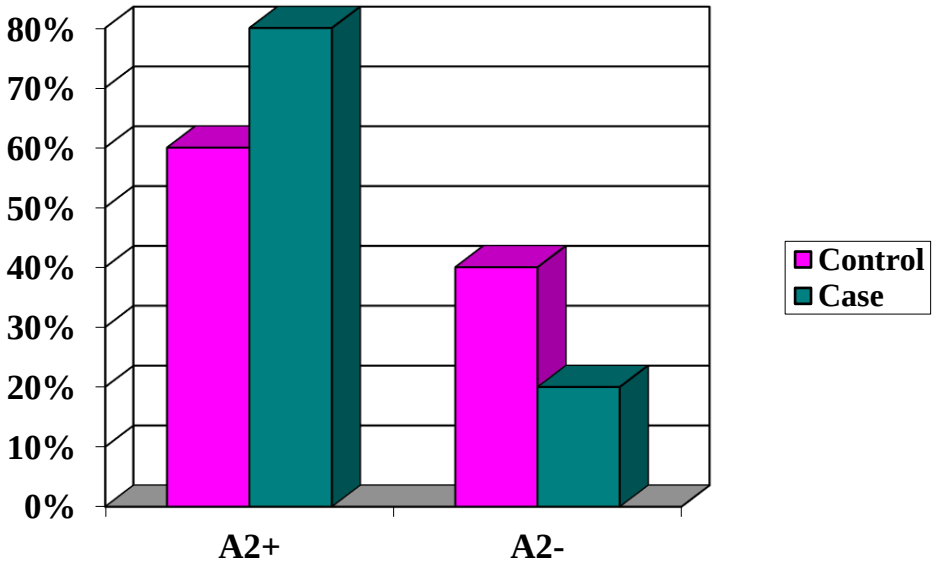

Fig-2: Allele frequencies of human CYP17 gene with Prostate Cancer

DISCUSSION

We evaluated the association between prostate cancer 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 prostate cancer (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), 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). 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. 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 prostate cancer. This connection is in line with the observation that a subset of prostate cancer has a genetic transmission [14]. It has been reported that the volume of prostate cancer is positively correlated with serum testosterone, estradiol, and estriol levels [15], therefore indicating imbalance of the androgen and estrogen resulting in development of prostate cancer. 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 prostate cancer.

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