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CYP17 Gene Polymorphism and its Association in North Indian Prostate Cancer Patients

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Abstract. *Background: The CYP17 gene codes for the cytochrome P450c17a enzyme, which mediates two key steps in sex steroid synthesis In this study, the association between CYP17 polymorphism and the risk of prostate cancer in comparison to benign prostatic hyperplasia (BPH) in a north Indian population was investigated. Patients and Methods: This study included 157 prostate cancer patients and 170 BPH patients as controls. A 451-bp fragment encompassing the polymorphic site was amplified by PCR and treated with the restriction enzyme MspA1. The undigested allele was recognized as A1 and the MspA1-digested variant allele was designated as the A2 allele. Results: Men with the A2/A2 CYP17 genotype had an increased risk of prostate cancer (OR=3.56; 95% CI=1.49-8.53; p=0.004) compared with those with the A1/A1 genotype. A significantly increased risk of prostate cancer was also found in smokers as well as non-vegetarians by four-fold as compared to their counterparts. There was a significant association between the CYP17 genotype and the tumour status (stage) of prostate cancer. The A2 allele showed a 1.90- (95% CI=1.09-3.32; p=0.02) and a 1.51- (95% CI=1.08-2.13; p=0.017) fold increased risk of prostate cancer in localized and metastatic prostate cancer cases respectively. Conclusion: The A2 allele of the CYP17 polymorphism is associated with an increased risk of prostate cancer and has a role in the development of prostate cancer in smokers and non-vegetarians.*

Genetic susceptibility in the progression of cancer is an important area to be explored, especially in hormone related carcinomas such as breast and prostate cancer (1). Prostate cancer is the sixth most common cancer in the world. Its incidence and mortality rates vary worldwide. Regardless of advancements in early detection and treatment methods, about 2,32090 new cases and nearly 30,350 deaths occurred in the United States in 2005 alone (2). The incidence and mortality rates of prostate cancer in Asian countries have been rapidly increasing. The increase in the incidence rates from 1973-1977 to 1988-1992 was estimated at 84% in Japan, 55% in Hong Kong, 44% in China and 16% in India (3). In 2001, 6,605 cases of prostate cancer were predicted out of a total 565,682 cases of all types of cancer in India. In developing countries such as India, 80% of victims already have disease at an incurable stage on diagnosis (4). Epidemiological studies have indicated the role of environmental, genetic and dietary factors in the progression of prostate cancer (5).

Androgens are crucial for the normal growth and development of the prostate gland and in maintaining its functional state in the adult and have been implicated in the development of prostate cancer. The increased levels of plasma testosterone and oestrogen are associated with an increased risk for prostate cancer (6). Testosterone is synthesized from cholesterol by a series of enzymatic reactions involving several of the cytochrome P450 enzymes such as CYP17. *CYP 17* is considered to be marker for prostate cancer susceptibility since it encodes the cytochrome P450c17 enzyme, which mediates two key reactions in steroid hormone biosynthesis: 17α-hydroxylation of pregnenolone and progesterone and 17,20-lysis of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone (7).

CYP17 is located on chromosome 10q24.3 and contains eight exons (7). A single nucleotide polymorphism (SNP) has been described in the 5' untranslated region, 27 bp downstream from the transcription start site and 34 bp upstream from the initiation of translation. It has been assumed that a thymidine (T) to cytosine (C) transition creates an additional recognition site (CCACT-CCACC) for

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the transcription factor Sp-1, therefore, altering the expression level of *CYP17* (8). Association studies have been conducted to investigate a possible effect of the *CYP17* polymorphism on the risk of the sporadic prostate cancer. However, the results have been inconclusive concerning the question of whether the wild-type allele (referred to as the A1 allele) or the altered allele (referred to as the A2 allele) can be considered as a risk factor. In the present study, both the alleles were studied in a north Indian population to investigate their influence on the progression of prostate cancer. The present study is a part of our efforts to determine the role of genetic polymorphisms in the risk of various carcinomas (9-18).

Patients and Methods

Blood samples from 157 north Indian males with prostate cancer were collected in sterile EDTA-K2 coated vaccutainer tubes (Becton Dickinson, San Jose, CA, USA). The samples were collected at the Departments of Urology of the Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh (India), the All India Institute of Medical Sciences (AIIMS), New Delhi and Government Medical College and Hospital, Patiala (Punjab) in north India. All the patients had been histologically diagnosed with carcinoma of the prostate confirmed with biopsy studies as well as prostate-specific antigen (PSA) test.

Control samples (170) were also collected amongst patients of the same urology departments who did not have any symptoms of cancer and had been diagnosed with benign prostate hyperplasia (BPH). The case and the control samples were matched for age. Although all the samples did not originate from the same hospital, all of them were from the general population of north India. No chemo- or radiotherapy had been given to any individuals before collecting the samples, which was confirmed from the hospital records as well as by the patients. The samples were collected by the clinical staff of the hospitals concerned. A detailed questionnaire designed by the Indian Council of Medical Research (ICMR) encompassing details of the disease diagnosis, family history, age, smoking and drinking status was completed at the time of collection of the samples. Besides this, the pathological grading and staging of the cancer was confirmed from the hospital record.

Blood samples were stored at –80˚C till extraction of DNA. The DNA was extracted by standard phenol-chloroform extraction (19). The polymorphism of *CYP17* was detected with restriction fragment length polymorphism.

A total volume of 50 μl of PCR reaction mixture consisted of 0.2 μg of genomic DNA, 0.5 μM of forward primer 5'-CAT TCG CAC TCT GGA GTC-3' and reverse primer 5'-GGC TCT TGG GGT ACT TG-3, 0.2 mM of each dNTPs, 2.5 mM of $MgCl_2$, 1x Taq Buffer and 1.5 U of Taq DNA Polymerase (MBI Fermentas, Vilnius, Lithuania) (20). The amplification was performed in a i-Cycler PCR system (Bio-Rad, Norwalk, USA) to obtain the PCR product of 459 bp.

The PCR included initial denaturation for five minutes followed by denaturation at 94˚C for 30 s and then annealing at 57˚C for 30 s and extension at 72˚C for 30 s for 30 cycles and finally extension at 72˚C for 5 minutes. For analysis of the polymorphism, 15 μl of PCR product was digested with 5 units of *MspA1* restriction enzyme (New England Biolabs, Beverly, MA, USA) at 37˚C for 3 h. The digested DNA was separated on 2.5% agarose gel stained with ethidium bromide for visualization under UV light (Bio-Rad). Digestion of the mutant genotype created 124 and 335 bp products, while an uncut product indicated the wild genotype of 459 bp. The heterozygous genotype showed all three bands (124, 335 and 459 bp).

Table I. *Demographic profile of the participants.*

Demographic factor	Cases (157)	BPH Cases (170)	OR (95% CI)
Mean age±S. Dev.	67.5±9.00	67.22±9.31	-
Urban	88 (56%)	97 (57.1%)	1.0
Rural	69 (44%)	73 (42.9%)	1.04 (0.67-1.61)
Occupation			
Sedentary worker	72 (45.9%)	82 (48.3%)	1.0
Manual worker	80 (50.9%)	84 (49.4%)	1.08 (0.70-1.68)
Industrial worker	05 (3.2%)	04 (2.3%)	1.42 (0.37-5.47)
Diet			
Vegetarian	71 (45.2%)	92 (54.1%)	1.0
Non vegetarian	86 (54.8%)	78 (45.9%)	1.43 (0.93-2.21)
Smoking status			
Non smoker	107 (68.2%)	126 (74.1%)	1.0
Smoker	50 (31.8%)	44 (25.9%)	1.22 (0.76-1.97)
Drinking status			
Non drinker	92 (58.6%)	100 (58.8%)	1.0
Drinker	65 (41.4%)	70 (41.2%)	1.05 (0.68-1.65)

Cases: prostate cancer; BPH: benign prostate hyperplasia; OR: odds ratio; CI: confidence interval.

Statistical analysis. Multivariate logistic regression was used to obtain the odds ratio (OR) estimate and 95% confidence interval (CI) for the main effects of the various demographic factors for each genotype. Adjusted ORs generated in this manner were evaluated for deviation from the expected null value on the additive or multiplicative scale. In addition, the interaction of these genes with environmental factors was evaluated for statistical significance with logistic regression. All the statistical analyses were performed using SPSS Professional Statistic software version 14 (SPSS Inc, Chicago, IL, USA).

Results

The study sample included 157 cases of prostate cancer and 170 of benign prostatic hyperplasia. The age of the patients ranged from 45 to more than 70 years. The representation from both rural (56%) and urban (44%) areas was found to be similar in prostate cancer and BPH patients. The number of industrial workers was found to be higher in cases (3.2%) as compared to BPH (2.3%). As many as 45.2% of patients were vegetarians, and 68.2% and 58.6% had never smoked or consumed alcohol respectively (Table I). The majority of the patients with prostate cancer (81%) had metastatic disease.

The genotypic distribution of the *CYP17 MspA1* polymorphism in both the prostate cancer cases and BPH is given in Table II. The frequency of A_1/A_1, A_1/A_2 and A_2/A_2

Table II. *Genotypic distribution of CYP17 gene polymorphism in prostate cancer cases and BPH patients.*

	A_1/A_1 (%)	A_1/A_2 (%)	A_2/A_2 (%)	OR[a] (95% CI)	OR[b] (95% CI)
BPH (170)	76 (45)	86 (55)	8 (5)	1.0	1.0
Cases (157)	48 (31)	91 (58)	18 (11)	1.67 (1.05-2.67)	3.56* (1.49-8.53)
Localised (30)	7 (23)	19 (63)	4 (14)	2.39 (0.97-5.90)	5.43* (1.46-20.24)
Metastaic(127)	41 (32)	72 (57)	14 (11)	1.55 (0.95-2.54)	3.24* (1.29-8.11)

*Indicates significant *p*-value; OR[a] odds ratio of A_1/A_2; OR[b] odds ratio of A_2/A_2; OR adjusted for age and smoking, drinking and non vegetarians.

was 31%, 58% and 11% in the prostate cancer cases and 45%, 55% and 5% in BPH. Men with the A_2/A_2 genotype had a highly significantly increased risk of prostate cancer (OR=3.56; 95% CI=1.49-8.53; p=0.004), whereas a marginal risk was found with the A_1/A_2 (OR=1.67; 95% CI=1.05-2.67) genotype. When the data were stratified with regard to tumour staging, a five-fold higher risk was observed in the cases with localized tumours, indicating its role in the development of prostate cancer (OR=5.43; 95% CI=1.46-20.24; p=0.01) in the initial stages. The risk, however, was the same in the metastasized cases (OR=3.24; 95% CI=1.29-8.11; p=0.01) when compared to total cases (OR=3.56; 95% CI=1.49-8.53; p=0.004).

The allele frequency of both the alleles in the prostate cancer cases and the BPH patients is given in Table III. The highest risk of prostate cancer was observed in the localized cases, with OR=1.90 (95% CI=1.09-3.32; p=0.02).

A significantly increased risk was also observed with the mutant genotype (A_2/A_2) of *CYP17* in smokers (OR=3.90; 95% CI=1.19-12.81; p=0.02) and non vegetarians (OR=4.30; 95% CI=1.27-14.53; p=0.02) (Table IV). About a two-fold increased risk of prostate cancer was also observed in the smokers with the heterozygous (A_1/A_2) genotype of *CYP17* (OR=1.87; 95% CI=0.94-3.72) and the combined heterozygous and mutant genotype groups (A_1/A_2, A_2/A_2) of *CYP17* (OR=2.15; 95% CI=1.13-4.09; p=0.019). The risk was increased to 2.3 (OR=2.26; 95% CI=1.19-4.28; p=0.01) and 2.5 (OR=2.48; 95% CI=1.30-4.63; p=0.005) in the heterozygous (A_1/A_2) as well as the combined (A_1/A_2, A_2/A_2) genotype of *CYP17* respectively in the non vegetarians.

Table III. *Allelic distribution of CYP17 gene polymorphism in prostate cancer cases and BPH patients.*

	A_1 (%)	A_2 (%)	OR (95% CI)
BPH (controls)	238 (70)	102 (30)	1.0
Prostate cancer cases	187 (59.5)	127 (40.5)	1.58* (1.15-2.19)
Localised cases	33 (55)	27 (45)	1.90* (1.09-3.32)
Metastatic cases	154 (60.6)	100 (39.4)	1.51* (1.08-2.13)

*Indicates significant *p*-value; OR adjusted for age and smoking, drinking and non vegetarians.

Discussion

To the best of our knowledge, the present study determined for the first time the allele frequencies and risk of prostate cancer in comparison to BPH in a north Indian population. There were two reasons for taking BPH as the control. Firstly, it would help in preventing any misdiagnosis of cancer and secondly, if any relationship were to be found with prostate cancer, then it would help in confirming the role of BPH in cancer progression (21).

It has been reported that the volume of BPH is positively correlated with serum testosterone, oestradiol and oestriol levels (22), therefore implicating thecomplex imbalance of the androgen and oestrogen environment in the development of BPH. A distinct sex-steroid hormone environment caused by the *CYP17* genotype presumably contributes to the development of BPH as well as prostate cancer (23).

In the present study, the frequency of the A_2/A_2 genotype was highest in the prostate cancer cases (11%) as compared to BPH (5%). The highest frequency among the prostate cancer cases (58%) and BPH (55%) was of the heterozygous genotype of *CYP17* (A_1/A_2). The presence of the A_2 allele significantly (p=0.005) increased the risk of prostate cancer.

A number of epidemiological studies have indicated that the variant allele (A_2) is associated with an increased risk of prostate cancer. Two studies from the United States (24) and Austria (25) have reported the role of the A_2 allele in the progression of prostate cancer. The role of the A_2 allele has been shown to increase the risk of prostate cancer by 2.4-fold in Japanese men (24). Similar results were also obtained by Kittles *et al*. (26) who found about a three-fold elevated risk of prostate cancer in African-Americans. In the Indian population, to our knowledge, only one study (14) concerning the *CYP17* gene polymorphism has been carried out and a non-significant marginally increased risk of prostate cancer for individuals carrying one copy of the A_2 allele (p=0.05) of the *CYP17* gene was found. The risk was increased (OR=2.81; 95% CI=1.06-7.40, p=0.03) in

Table IV. *Risk of prostate cancer in smokers and non vegetarians due to CYP 17 polymorphisms.*

Genotype	Non-smokers *vs*. Smokers			Vegetarians *vs*. No vegetarians		
	Cases	BPH	OR (95% CI)	Cases	BPH	OR (95% CI)
A_1/A_1	34	59	1.0	23	44	1.0
A_1/A_2	27	25	1.87 (0.94-3.72)	52	44	2.26* (1.19-4.28)
A_2/A_2	09	04	3.90* (1.19-12.81)	09	04	4.30* (1.27-14.53)
			90			
$A_1/A_2/A_2/A_2$	36	29	2.15* (1.13-4.09)	61	48	2.48* (1.33-4.63)

*indicates significant *p*-value; OR adjusted for age and drinking.

individuals having two A_2 alleles (14). Wadelius *et al*. (27) as well as Habuchi *et al*. (28) found significant elevation of prostate cancer due to the A_1 allele. When all the studies in Caucasian populations were compared under a meta-analysis, no association was found with any of the genotypes in the development of prostate cancer (29).

When the data were stratified with regard to tumour staging, a five-fold significantly increased risk was observed in the cases with localized tumours, indicating its role in the development of prostate cancer, while the risk decreased to three-fold in the metastasized cases. This result may have been due to the small sample size of the localized cases, which, therefore, limited the power to detect moderate effects of the potential risk genotype.

The present study was also performed to study the role of smoking as well as dietary habit along with *CYP17* gene polymorphism. The significantly increased risk of prostate cancer of 3.9-fold (p=0.02) was observed in those prostate cancer patients who smoked during their lifetime. The risk was also increased to 4.3 (p=0.02) in those consuming a non vegetarian diet (Table IV). According to Gann *et al*. (6), smoking affects the blood concentration of various hormones. It is known that high testosterone and a low level of oestrogens and sex hormone-binding globulin may increase the chances of prostate cancer. From various studies, it has also been found that levels of hormones, such as cortisol and androstenidione, the total plasma testosterone and sex-binding globulins produced by the adrenal gland are statistically significantly higher in smokers than non-smokers (30, 31) but the full impact of these increases in hormonal levels is not yet established. Similarly, the role of non vegetarian food, found to be rich in carcinogenic elements has also been studied.

The present findings suggest that the *CYP7* genotype is involved in distinct pathways of cellular growth of the prostate gland. Besides this, a non vegetarian diet and smoking has augmented the risk of prostate cancer along with the mutant genotype of *CYP17*. In conclusion, *CYP17* gene polymorphism may be significantly associated with a risk of prostate cancer in this north Indian population.

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