Prostate Cancer Risk in Relation to CYP3A4 and CYP3A5 Genotypes in the Bangladeshi Population

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Received: January 03, 2016; Accepted: January 24, 2016; Published (web): January 31, 2016

ABSTRACT: Genetic polymorphism on CYP3A4 and CYP3A5 gene and their associational susceptibility to prostate cancer was studied on Bangladeshi population, considering the importance of CYP3A4 and CYP3A5 gene in detoxification of xenobiotics from physiological territory. In this case control regulated study, we focused on two allelic variants CYP3A4 rs2740574 (CYP3A4*1B) and CYP3A5 rs776746 (CYP3A5*3) applying Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP). Associational risk on prostate cancer was estimated as odds ratio (OR) and 95% confidence interval (CI) using unconditional logistic regression models. An elevated prostate cancer risk was found with heterozygous, mutant and heterozygous plus mutant variants of CYP3A4*1B which is not statistically significant ($p>0.05$), whereas a significant association was found with heterozygous, mutant and heterozygous plus mutant variants of CYP3A5*3 (OR =4.36, 95%CI = 1.53 to 12.38, P =0.003; OR =3.85, 95%CI = 1.19 to 12.43, P = 0.017 and OR =4.13, 95%CI = 1.84 to 9.28, p =0.0006 respectively). The findings signposted a significant association of CYP3A5*3 gene and nullify the association of CYP3A4*1B gene's polymorphism with prostate cancer risk in Bangladeshi subject.

Key words: CYP3A4, CYP3A5, prostate cancer, Bangladeshi population

INTRODUCTION

Prostate Cancer (PCa) has been the most common cancer in the world for several decades, and by 2012, the estimated cases were 1112,000 representing the 15% of the cancers diagnosed in men whereas the estimated deaths were 307,000 and it was the fifth leading cause of death from cancer in men (6.6% of the total men deaths).[1] The estimated incidence and mortality of prostate cancer in Bangladesh were 923 and 717 in 2012 and that will be 1810 and 1342 in 2030, respectively.[1] PCa is one of the leading causes of cancer death in men, and both genetic and environmental factors have been shown to be important for the development of this disease.[2-5] Prostate cancer can grow in an androgen-dependent pathway by the activation of androgen and about 95% of prostate tumors develop from the luminal cells. There are three distinct glandular regions in the human prostate gland such as peripheral, central, and transitional zone comprising 70%, 30%, and 5% of the normal glandular prostate mass respectively.[6] Large percentage of PCa is due to the presence of common polymorphisms whereas family history is responsible only 5-10% of prostate cancers.[7,8] Recently, researchers have focused on the role of genes involved in the metabolism,

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biosynthesis, and regulation of androgens in the occurrence and progression of prostate cancer. The CYP family of enzymes plays a vital role in a wide variety of metabolic pathways involving both endogenous and exogenous substances[9] and the metabolism of steroids, as well as environmental xenobiotics may have significant influence on prostate cancer.[7-9] CYP3A4 belongs to the cytochrome P-450 supergene family is involved in the metabolism and most likely the deactivation of testosterone.[10,11] It has been reported that, the genetic variant in the 59 regulatory region of the CYP3A4 gene (A to G transition at position 2293 from the ATG start site) is associated with prostate cancer.[12] CYP3A4 is involved in oxidation of testosterone to 2β-, 6β-, or 15β-hydroxytestosterone.[10] It has been postulated that the presence of the CYP3A4*1B allele decreases the amount of CYP3A4 protein, leading to a reduction of testosterone metabolism and therefore more availability of testosterone for conversion to dihydrotestosterone, the most potent androgen affecting the growth and differentiation of prostate cells.[13] However, several studies failed to link up the activity of the CYP3A4*1B with PCa.[14-17] It was found that Caucasian carriers of CYP3A4*1B had a higher Tumor-Node-Metastasis stage and Gleason grade than the non-carriers.[18-20] On the other hand the CYP3A5*1 variant was inversely associated with prostate cancer, especially among Caucasians with less aggressive disease.[20] One researcher reported that minor allele frequencies of Korean population were similar with those of the Japanese and Han Chinese populations, whereas distinct differences were found from European-Americans or African-Americans. Among the pharmacogenetic markers frequencies of CYP3A4*1B (rs2740574) and CYP3A5*3C (rs776742) in Asian groups were different from those in other populations.[21] This study was designed to find out the relationship of CYP3A4rs2740574 and CYP3A5rs776746 polymorphisms with Bangladeshi prostate cancer patients.

MATERIALS AND METHODS

Study design. This case-control study was conducted on 100 prostate cancer patients and 100 healthy volunteers matched by age, sex and smoking status. Prostate cancer patients were recruited from Ahsania Mission Cancer and General Hospital (AMCH), Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Patients were histologically diagnosed with prostate cancer between the period of January 2011 and December 2012. The Institutional Review Boards of the mentioned hospitals approved the study design. The clinical characteristics, including Gleason grade, PSA, and TNM stage, as well as other potentially important factors, such as age at diagnosis and family history of prostate cancer were recorded during the study. Tumor grade was determined according to the Gleason system[22-24] whereas the tumor stage was determined after review of the microscopic sections of the specimen.[25] All cases were histologically confirmed and underwent radical prostatectomy, transurethral resection of the prostate, or prostatic biopsy. Smoking information, demographic characteristics, and lifestyle factors were collected through interviews by trained nurses in the presence of expert physicians. Prostate cancer cases had a history or evidence of any other severe diseases like cardiovascular disease, kidney disease; previous cancer and metastasized cancer were excluded from the study. Controls were not relatives to the patients and no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations or any acute or chronic diseases based on medical history, clinical examination and laboratory investigation (hematology, blood biochemistry and urine analysis). The study was conducted in accordance with the declaration of Helsinki and its subsequent revisions.[26]

Genomic DNA isolation and genotyping. Venous blood (3 ml) was collected from all cases and controls in sterile tubes containing EDTA-Na_2 and stored at -80 °C until DNA extraction. Genomic DNA was extracted from blood samples of all

subjects according to our previous method.[27-30] For genotyping, PCR-RFLP was employed due to its affordability, ease of use and reliability. CYP3A4*1B, CYP3A5*3 were genotyped by previously described method.[27,30] The primers, PCR conditions, and restriction enzymes are presented in table 1. A 25µl PCR reaction volume was used, containing 1 µl of genomic DNA (50–70 ng/µl), 5 µl of 5x GoTaq reaction buffer, 4 µl of $MgCl_2$ (25 mM), 2 µl of dNTPs (2.5mM), 1 µl of each primer (10 µM), 0.1 µl of GoTaq DNA polymerase (5 U/µl) (Promega Corporation, USA), and 10.9 µl of nuclease free water. PCR products were analyzed on a 2% agarose gel by staining with ethidium bromide. After PCR amplification, 20 µl PCR products were digested (overnight at 37 °C) with approximately 2 units of MboII and RsaI for CYP3A4*1B and CYP3A5*3 respectively. Electrophoresis was done for restriction enzyme digested products using 3% agarose gel in 1x TAE buffer or 10% polyacrylamide gels in 1x TBE buffer. Samples found heterozygous and mutant homozygous were analyzed twice and subject to direct sequencing by using our previous reported method.[27-30]

Table 1. Primers, PCR conditions, restriction enzymes (RE) and expected DNA fragments on digestion to genotype the selected polymorphisms.

Allele	Primer sequence	PCR (35 cycles)	RE	DNA fragments
CYP3A4*1B	FP 5'-GAATGAGGACAGCCATAGAGACAAGGGGA-3'RP 5'-CCTTTCAGCTCTGTGTTGCTCTTTGCTG-3'	94 ºC 1 min 57 ºC 1 min 72 ºC 1 min	MboII	AF 385 NH 175, 169, 41 HE 210,175,169,41 MH 210, 175
CYP3A5*3	FP 5'-CCTGCCTTCAATTTTTCACT-3' RP 5'- GTCCAAACAGGGAAGAGGT-3'	94 ºC 1 min 59 ºC 1 min 72 ºC 1 min	RsaI	AF 196 NH 102, 74, 20 HE 102, 94, 74, 20 MH 102, 94

Statistical analysis. Distributions of demographic variables were compared between cases and controls using $\chi 2$- tests and two-sided unpaired t-tests. Genotype and allelic frequencies were reported as percentage and the distribution of genotype frequency was also compared by $\chi 2$- test. Unconditional logistic regression was used to estimate crude odds ratio (OR) and their 95% confidence intervals (CIs) using the statistical software package SPSS version 20.0 (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Cases and controls characteristics and histological subtype of prostate cancer. The distributions of demographic characteristics and clinical data among study subjects were summarized in table 2. The related factors such as age and smoking history of cases and controls were compared to confirm that the observed effects were solely due to the genotype frequency. There were no significant differences in mean age ($p = 0.776$) and smoking status ($p = 0.788$) between the two groups.

Among 100 cases, the histological subtypes of prostate cancer were 19% of weak anaplasia (Gleason 2-4), 18% of moderate anaplasia (Gleason 5-6) and 63% of marked anaplasia (Gleason 7-10). The mean and range of prostate specific antigen (ng/dl) is 56 and (20-176) respectively (Table 2).

Smoking status. The observed never smoking rate was 7% in the cases and 8% in controls. Among the smokers 65% and 21% were current and ex-smokers in cases and were 69% and 20% in controls, respectively. The rates of chewing tobacco were 7% and 3% in cases and controls respectively. There is no significant difference between current smoker, ex-smoker, never smokers and chewing tobacco groups between cases and controls ($p = 0.788$) (Table 2).

CYP3A4 polymorphisms. The genotypes of CYP3A4*1B in both cases and controls were presented in table 3. There was no significant difference found among the genotypes of cases and controls (p=0.356). Compared with the wild type genotype, patients carrying heterozygous, mutant homozygous and combined heterozygous plus mutant homozygous of CYP3A4rs2740574 showed increased risk of prostate cancer (OR = 2.46, 95% CI = 0.62 to 9.81, p = 0.202; OR = 2.11, 95% CI = 0.19 to 23.67, p = 0.545 and OR = 2.37, 95% CI = 0.71 to 7.98, p = 0.162, respectively) and that were not statistically significant.

Table 2. Distribution of demographic variables of the prostate cancer patients and controls.

Variables	Cases (n=100) (%)	Controls (n=100) (%)	P-value
Age (years)			
Mean age, n (±SD)	66 (±2.6)	66 (±2.7)	0.7[a]
Range	63-72	62-72	
Smoking status, n (%)			
Current smoker	65	69	-
Ex-smoker	21	20	
Chewing tobacco	7	3	
Never smoker	7	8	
Total tobacco user	93	92	0.7[b]
Total tobacco nonuser	7	8	
Histological type, n (%)			
Weak anaplasia: Gleason 2-4	16	-	-
Moderate anaplasia: Gleason 5-6	18		
Marked anaplasia: Gleason 7-10	63		
Prostate Specific Antigen (ng/dl)			
Mean (±SD)	56 (±31.1)	-	-
Range	20-176		

[a] Unpaired t test, [b] χ^2 test.

CYP3A5 polymorphisms. The genotypes of CYP3A5 in both cases and controls were presented in Table 3. Significant difference (p=0.001) among the genotype frequencies of cases and controls was found in the cases and controls. Compared with the wild genotype, patients carrying heterozygous, mutant homozygous and combined heterozygous plus mutant homozygous of CYP3A5rs776746 showed significant association with the risk of prostate cancer (OR = 4.36, 95% CI = 1.53 to 12.38, p = 0.003; OR = 3.85, 95% CI = 1.19 to 12.43, p = 0.017 and OR = 4.13, 95% CI = 1.84 to 9.28, p = 0.0006 respectively).

In this study, we tried to find out the association between CYP3A4*1B and CYP3A5*3 polymorphisms and prostate cancer risk in Bangladeshi population. It is the first of such kind of epidemiological study on Bangladeshi population. We focused on prostate cancer, as it is one of the most prevalent causes of cancer related death in Bangladesh. There were no significant differences in mean age (p = 0.776) and smoking status (p = 0.788) between the two groups (Table 2).

Our results revealed that, the genotype of CYP3A4*1B variant has increased risk but not significantly associated (p>0.05) with prostate cancer (Table 3). Unfortunately, we had limited ability to obtain statistical significance because of small sample size low frequency of this allele in the study population. Our finding is consistent with some studies conducted on other Asians[31-33] and similar finding was also observed in non- Asians

population.[13,18,19] Several researchers found an inverse association between CYP3A4*1B and prostate cancer risk.[34,35] One study conducted on South African White and Mixed Ancestry men where a positive association ($p < 0.05$) was observed between the AG and AG/GG genotypes of CYP3A4rs2740574 and PCa.[36] A number of researchers investigated the effects of CYP3A4*1B in protein expression or function[14,16,37-41] and the majority of the studies reported elevation of CYP3A4 levels in the presence of G carrier genotypes. However, most of the authors reported a small magnitude of association of CYP3A4 and CYP3A5 with prostate cancer prognosis[37] and almost all studies have reported consistent elevations in expression associated with CYP3A4*1B in the range of 20-200% increase over the consensus CYP3A4*1A.[34] In this case control study we found a significant elevated risk of prostate cancer with CYP3A5*3 genotypes that is consistent with one previous report.[42] Some researchers also found opposite result that is may be due to the uniqueness of the ancestry of the Indian subcontinent.[36,42,43] This study is on the Bangladeshi population that was also a part of Indian subcontinent earlier and no study was yet reported with the mentioned genes on native Indian prostate cancer patients.

Table 3. CYP3A4*1B and CYP3A5*3 genotypes and allelic frequencies among prostate cancer cases and controls.

	Cases (%)	Controls (%)	OR	95% CI	P
CYP3A4*1B genotype					
*1A/*1A	91	96	1	--	--
*1A/*1B	7	3	2.46	0.62 to 9.81	0.202
*1B/*1B	2	1	2.11	0.19 to 23.67	0.545
*1A/*1B+*1B/*1B	9	4	2.37	0.71 to 7.98	0.162
CYP3A4 allele					
CYP3A4*1A	189	195	1	--	--
CYP3A4*1B	11	5	2.27	0.77 to 6.66	0.135
CYP3A5*3 genotype					
*1/*1	71	91	1	--	--
*1/*3	17	5	4.36	1.53-12.38	0.003
*3/*3	12	4	3.85	1.19-12.43	0.017
*1/*3+*3/*3	29	9	4.13	1.84-9.28	0.0006
CYP3A5 allele					
CYP3A5*1	159	187	1	--	--
CYP3A5*3	41	13	3.7	1.92-7.17	0.0001

OR= Odds ratio, CI= Confidence interval

CONCLUSION

We investigated the effect of CYP3A4*1B and CYP3A5*3 as risk factors for the development of prostate cancer among Bangladeshi population. Our observation stated that CYP3A4*1B polymorphism is not significantly associated with increased risk for prostate cancer whereas CYP3A5*3 is significantly associated with the risk of prostate cancer in the study subject of Bangladeshi population.

ACKNOWLEDGMENTS

The authors are thankful to all the staffs, nurses, and physicians of Ahsania Mission Cancer and General Hospital (AMCH), Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh and the participants of this study.

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