Original Research

Association of vitamin D receptor gene polymorphisms with benign prostatichyperplasia in North Indian population

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ABSTRACT

Background: The study aimed to investigate the association between single nucleotide polymorphism (SNP) of the vitaminD receptor (VDR) gene(FokI,ApaI and TaqI) and benign prostatic hyperplasia (BPH) in North Indian men. **Methods:** The DNA wasextracted from the blood of 120 BPH patients and 120healthy age-matched controls and it was subjected to PCR followed by restriction fragment length polymorphism analysis. The odds ratio (OR) of having agenotype and developing BPH was calculated and the alleleswere designated risk-bearing or protective. **Results:**It was observed that Tt and Aa genotype was significantly low in BPH group as compared to healthy controls.No significant association was found with FokI polymorphism. **Conclusion:** This study suggests the protective role of heterozygous genotypes of TaqI and ApaI polymorphism against the development of BPH.

INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a prevalent urological condition affecting aging males, characterized by non-cancerous enlargement of the prostate gland. It is one of themostcommon diseases causing lower urinary tract symptoms, in middle-aged and elderly men and a significant worldwide public health issue.[1]Changes in the molecular mechanism regulating programmed cell death and cell proliferation underlie the abnormal growth of the gland leading to BPH.Estimates of BPH prevalence range from 40 to 50% at 50 years of age to as high as 80% for men aged 70 years.[2]Also, withincrease in the population andaging, BPH contributes to a greater burden of health care and financial expenditure.[3] Symptoms of BPH includes hesitancy,urgency, frequency, post-void dribbling and nocturia which results in declining quality oflife.[4]The etiology of BPH is not completely understood but it seems to be multifactorial and endocrine controlled.Androgens, estrogens, stromal epithelial interactions, growth factors, and neurotransmitters may play a role, either singly or in combination, in the etiology of the hyperplastic process.[5]Some studies have suggested a genetic predisposition and some have noted racial differences.[4]But BPH lacks established genetic markers for determining disease susceptibility.Vitamin D is a secosteroid hormone modulating calcium homeostasis through actions on kidney, bone and intestinal tract. The human prostate expresses the vitamin D receptor (VDR), and calcitriol inhibits the in vitro growth of both epithelial and stromal BPH cells. In addition, calcitriol exhibits antiproliferative and prodifferentiatingactivities in malignanat prostate cell lines and in some in vivo models of prostate cancer.[6-9]Low vitamin Dlevel is regarded as an independent risk factor for development of BPH .[10]Studies have shown thatVDRplayed an importantrole in the development of BPH.[11] Gene encoding VDR is located on the chromosome 12q13 and is a memberof the nuclear hormone receptor superfamily which effectsthe function of genes that

are involved in cell regulation,growthand immunity.[12]To date, several single nucleotide polymorphisms (SNP) of the VDR gene have been described using different restriction enzymes such as ApaI, BsmI, TaqI, FokI, Tru9I, and EcoRV.[13]Various studies have been done exploring their role inthe development of the BPH.[14-17]However, there is no conclusive evidence to establish as relationship between VDR polymorphismsand occurrenceof BPH.In view of the fact that BPH progression may results in harming outcomes requiring surgical treatment like transurethral resection of the prostate,it is clinically important todevelop a biomarker that can be used for predicting risk of development of BPH. There are very few studies based on Indian population that established link between VDR gene polymorphism and BPH. Hence, we investigated the association between VDR gene polymorphisms namely FokI, TaqI and ApaI and its association with the risk of BPH in Indian cohort.

MATERIALS AND METHODS

Study population: Thisstudy was conducted at the tertiary care hospital in North India, in the department of Biochemistry and with the association of department of Urology. Clearance from Institutional Ethical Committee was obtained preceding the study. Cases included 120 newly diagnosed patients of BPH. The patients presented with lower urinary tract symptoms with moderate to severe American urological association (AUA) symptom score. Digital rectal examination (DRE) was done which revealed the enlargement of prostate. The volume of prostate of all patients was evaluated by transrectal ultrasound (TRUS). The patients having serum prostate serum antigen (PSA)level of >4ng/ml were screened for TRUS guided prostatic true-cut biopsy to rule out prostate cancer. All patients in the study have signed an informed consent for participation. A total of 120 age matched normal healthy controls were enrolled from patients visiting the hospital for trivial medical or surgical difficulties after informed consent. All controls have normal PSA level and absence of symptoms suggestive of BPH, malignancy or other related disease. There was no statistically significant difference between mean age of cases and controls ($p > 0.05$).

DNA Extraction: Blood samples were collected in vials containing EDTA K_2 anticoagulant and stored at -80°C till further analysis. Total genomic DNA was isolated from whole blood using the method described by Daly AK et al.,.[18]

PCR Amplification: The required region of VDR gene from the genomic DNA was amplified by polymerase chain reaction in MJ Research PTC-100™ (Peltier Thermal Cycler). Primers used were as follows:

Primers for FokI: (Harris SS et al.,)[19]

Forward: 5'- AGC TGG CCC TGG CAC TGA CTC TGC TCT-3'

Reverse: 5'- ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'

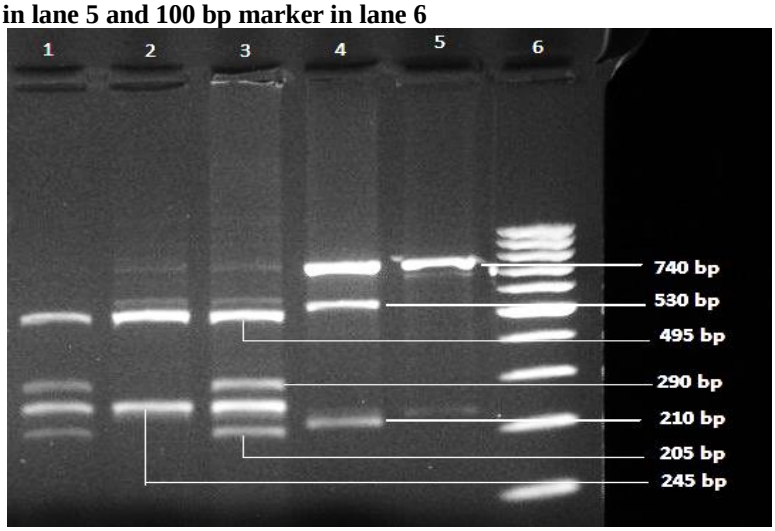
Primers for TaqI and ApaI: (Riggs BL et al.,)[20]

Forward: 5'- CAG AGC ATG GAC AGG GAG CAA-3

Reverse: 5-GCA ACT CCT CAT GGC TGA GGT CTC-3

RFLP Analysis of SNP: For FokI, PCR product of 265 bp was gained and confirmed using a 2% agarose gel. The PCR product was digested with Fok-I restriction enzyme obtained from New England Biolabs (NEB). The FF genotype lacked a Fok-I site and showed only one band of 265 bp. The ff genotype producedtwo fragments of 196 and 69 bp. The heterozygote showedthree fragments of 265, 196 and 69 bp, designated as Ff. (Figure 1)

Figure 1: Agarose gel picture of electrophoresis pattern of restricted enzyme digested PCR products showing Heterozygous (Tt) in lane 1 and 3, Homozygous (TT) in lane 2, Heterozygous (Aa) in lane 4, Homozygous (AA) in lane 5 and 100 bp marker in lane 6



In case of TaqI polymorphism, the PCR product of 740 bp was attainedand digested with Taq-I restriction enzyme from NEB. Taq-I digestion disclosedone obligatory restriction site, the homozygous TT (absence of the specific Taq-I restriction site) produced bands of 245 bp and 495 bp. The homozygous ttdisplayed205, 245, 290 bp and the heterozygous Tt presented495, 205, 245, 290 bp fragments.For ApaI polymorphism, PCR product of 740 bp was digested with Apa-I restriction enzyme. The lack of ApaI site exhibited only one band at 740 bp and designated as AA, whereas presence of restriction site indicatedtwo fragments at 210 and 530 bp and designated asaa. The heterozygote displayed three fragments of 740, 210 and 530 designated as Aa.

Statistical Analysis: Data were statistically analysed using Graph Pad Prism (8.0version) software. The Hardy–Weinberg equilibrium was tested to compare observed genotype frequencies withexpected ones using the Chi-square ($\chi 2$) test.Fisher's exactprobability test was used to evaluatedifferences in genotypeand allele prevalence between the groups. Odds ratioswith *p*values and 95% confidence intervals were calculatedusing the contingency table.All *p* values weretwo-sided and were considered as significant when being lessthan 0.05.

RESULTS

120 diagnosed patients of BPH were included in the studyrecruited from department of Urology at a tertiary hospital. Their mean age was 65.7 years. One hundred twenty age and sex matched healthy volunteers were recruited in the control group. Their mean age was 65.8 years. Table 1 shows the comparison of VDR gene polymorphism(TaqI, ApaI and FokI) in both the groups.The distribution of SNP was in conformity withHardy-Weinberg equilibrium for both groups.Frequencies of single allele are shown in Table 2. Frequency of single "*a*"allele in BPH patients was statistically significant when compared with control group.However, analysis of other single alleles i.e., *t* and *f* did not have any significant association. In FokI polymorphism study, no statistically significant difference was found between two groups. It was observed that *Tt* and *Aa* genotype was significantly low in BPH group when compared to healthy controls.

Table 1:Comparison of VDR gene polymorphism between BPH and Control Group.

Polymorphism	Genotype	BPH n (%)	Control Group n (%)	Odds Ratio	95% CI	p value
TaqI	TT	64 (53.3)	40 (33.3)	1 (ref)		
	Tt	36 (30)	74 (61.7)	0.3041	0.1139—0.8113	0.017*
	tt	20 (16.7)	6 (5)	2.083	0.4311—10.07	0.4485
ApaI	AA	76 (63.3)	40 (33.3)	1 (ref)		
	Aa	32 (26.7)	68 (56.7)	0.2477	0.0916—0.669	0.008*
	aa	12 (10)	12 (10)	0.5263	0.115—2.411	0.4783
FokI	FF	88 (73.3)	86 (71.7)	1 (ref)		
	Ff	28 (23.3)	30 (25)	0.9121	0.324—2.565	1.00
	ff	4 (3.3)	4 (3.3)	0.9773	0.0839—11.39	1.00

*p<0.01

Table 2:Distribution of allelic frequency among cases and controls.

Polymorphism	Allele	BPH n (%)	Control Group n (%)
TaqI	T	82 (68.3)	**77** (64.2)
	t	38 (31.7)	43 (35.8)
ApaI	A	92 (76.7)	74 (61.7)
	a	28 (23.3)*	46 (38.3)
FokI	F	102 (85)	101 (84.2)
	f	18 (15)	19 (15.8)

*p<0.05

Table 3: Worldwide study of genetic variants of VDR and association with riskofBPH.

Sr.No.	Study	Country	No. of cases	Polymorphism	Association
1	Manchanda et al[14]	India	160	FokI, TaqI and BsmI	S (with TaqI and BsmI)
2	El Ezzi et al[15]	Lebanon	68	FokI, TaqI, ApaI and BsmI	S(with ApaI and BsmI)
3	Ruan L et al[16]	China	200	FokI	S
4	Nunes et al[17]	Brazil	41	FokI, TaqI, ApaI and BsmI	S (with TaqI)
5	Huang et al.[21]	Taiwan	189	FokI	NS

S: Significant; NS: Non-significant.

DISCUSSION

Incidence of benign prostatic hyperplasia is rising worldwide as the life expectancy is increasing. Role of vitamin D receptor polymorphism in the development of BPH has been studied by few researchers but results are inconclusive specially in the Indian context (table 3).One reason of such inconsistency may be that the frequencies of VDR gene polymorphisms differ among different racial and ethnic groups. Therefore, present study was designed which include 120 patients of BPH and one hundred twenty age and sex matched healthy controls. We found that patients of BPH possesses *Tt* and *Aa* genotype at statistically low proportion when compared with healthy controls. Also, these patients have low single allele "*a*" of ApaI SNP. This suggest that carriers of single *t* allele and single *a* allele have lesser risk of having BPH. This study does not find any significant association with FokI polymorphism.A study in Japanesepopulation[22]which included 209 BPH cases and 128 controls did not find any significant association between TaqI and ApaI polymorphism and risk of BPH. The study carried out in India by Manchanda et al.,[14] showed significant difference in TaqI genotype but not with FokI genotype. The frequency of *tt*genotype in their study was 10% while our study showed it 16.7%. While 2% of the patients in above mentioned study have *ff* genotype, our study showed it to be 3.3%.A meta-analysis carried out by Li Ruan[23] involving 1539 Benign prostatic hyperplasiacases and 1915 controls found a significant positive association with BPH risk only in the TaqI SNP.Moreover, a high degree of heterogeneity was observed in the subgroup analysis of theassociation between FokI variants and BPH risk.However another meta-analysis[24]found no significant association between VDR gene polymorphism and BPH.A study involving Chinese population[16]indicated that the *f* alleles of the VDR gene FokI SNP associated with the progression of BPH. Thus, the majority of the studies on VDR gene polymorphisms and risk of development of the BPH revealed inconsistent results. This might be due to many factors like racial and ethnic differences, a possible polygenic basis for the disease, unevenness in study plans, and environmental effects on BPH development[25,26].

The most commonly used biochemical marker universally for diagnosis of BPH is the prostate specific antigen (PSA), but unfortunately PSA level increases only in advanced stages of clinical BPH[27] and also in case of prostate cancer (Pca). Thismakes it difficult at the gray zone of PSA (between 4 and10 ng/ml) to distinguish between BPH and Pca without an invasive method i.e.,prostate biopsy[28]. These facts stress on the development of novel molecular marker to establish the diagnosis of BPH. Results of this study can assist to use VDR gene polymorphism as genetic marker for predicting risk of BPH. However, a greater number of samples should be examined andmore studies need to be undertaken on different population and ethnic groups.

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