RESEARCH COMMUNICATION

Association of Genetic Variants of the Vitamin D Receptor (VDR) Gene (Fok-I, Taq-I & Bsm-I) with Susceptibility of Benign Prostatic Hyperplasia in a North Indian Population

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Abstract

Several genetic studies worldwide have recommended VDR as candidate gene for determining risk of benign prostate hyperplasia (BPH). We investigated the association between VDR gene polymorphisms and the risk of BPH in an Indian male population. Three polymorphic sites of VDR gene, viz., Fok-I, Taq-I and Bsm-I were genotyped in 160 BPH patients and 160 controls. Logistic regression models were used to determine the genetic effects using SPSS statistical software. A statistically significant association between VDR genotype (Taq-I and Bsm-I) and BPH (p=0.02 & 0.03) was obtained. In exploratory analyses, we also examined the association with responder and non-responder subgroups of patients for association of VDR (Taq-I) genotype with drug responsiveness. Our results established that Taq-I and Bsm-I genetic variants of VDR gene influence susceptibility BPH in Indian population. VDR genotypes specifically, Taq-I polymorphic variant is significantly associated with the improvement of BPH patients with standard drug therapy.

Keywords: VDR - polymorphism - PCR-RFLP - benign prostatic hyperplasia

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Introduction

BPH is most commonly encountered non-neoplastic prostatic disease. Despite its high prevalence and major impact on quality of life of patient, the aetio-pathogenesis and risk factor of BPH are not clear. The only risk factor of BPH established in several independent and longitudinal studies is aging. BPH lacks established genetic markers for determining disease susceptibility.

Vitamin D primarily known to be involved in calcium homeostasis also regulates growth and differentiation of diverse types of cells through specific receptor, the VDR (Feldman et al., 2001). Interaction of vitamin-D with VDR is thought to influence androgen receptor activation and the development of BPH (Blazer et al., 2000). Vitamin D3, the activated form of vitamin D and some of its analogues have been described as potent regulators of cell growth and differentiation of isolated epithelial cells derived from patients with BPH (Crescioli et al., 2000). VDR agonists have been shown to be useful in treating BPH patients (Adorini et al., 2000). While others reported that in the absence of testosterone, 1,25-D might exert a growth-promoting effect on the prostatic stroma in vivo (Konety et al., 1996). Recently it was found that a low vitamin D level is linked to increase prostate volume and a low vitamin D level is an independent risk factor for BPH (Hammarsten et al., 2006). Also VDR expression in normal prostate declines with age and prostate hyperplasia is also strongly associated with age (Krill et al., 2001).

Several polymorphisms have been identified in the VDR gene, and their functional significance and potential effects on susceptibility of various diseases have been investigated (Zamuda et al., 2000). VDR polymorphisms have already been reported to be associated with CaP (cancer prostate) (Habuchi et al., 2000). It is known that BPH may not always lead to CaP and even independent co-existence of same condition was reported indicating possible differences in etiology and pathway of progression. However, it was also not well established that specific genetic events in BPH could not lead to CaP.

Also there are some studies indicating significant association of VDR polymorphisms with BPH, but it is not unequivocal (Table 1). One reason of such inconsistency may be that the frequencies of VDR polymorphisms genes also differ among different racial and ethnic groups (Bid et al., 2005). Until similar studies of good statistical strength been conducted worldwide, it would not be possible to conclude without bias. The association between

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VDR genotypes and BPH in Indian patient is currently not known. Therefore, the purpose of this study was to investigate VDR gene polymorphism and its association with risk of BPH in India. In addition, we went for analyzing association of specific VDR polymorphism with response pattern of patient against standard combination therapy.

Materials and Methods

Study Populations and Sample Processing

The present study comprised of 160 BPH patients (mean age=64.35+9.14 years) enrolled from the Department of Urology, CSMMU, Lucknow during the period of July 2005-October 2007. This study was conducted with prior clearance from the ethical committee of CSMMU (Chatrapati Shahuji Maharaj Medical University), Lucknow.

The clinical presentations of patient were of LUTS (lower urinary tract symptoms) with moderate to severe AUA (American urological association) symptom score. DRE (digital rectal examination) revealed the enlargement of prostate. The prostate volume of all patients was assessed by transrectal ultrasound (TRUS). The patients with serum PSA (prostate serum antigen) of >4ng/ml were subjected for TRUS guided prostatic true-cut biopsy to rule out CaP. A total of 160 age matched normal healthy controls (mean age=62.9+9.7 years) were recruited from patients visiting the hospital for minor medical or surgical problems after inform consent. All were screened for normal PSA level and absence of symptoms suggestive of BPH, malignancy or other related disease.

The subjects were treated as per standard follow up protocol. Subjects showing improvement in Qmax (maximum flow), reduction in AUA score and post void residue within 6 months of combined therapy (5α reductase inhibitors + β adrenergic blockers) were categorized in Group I (responder) and those who failed to improve the parameters were categorized as Group II (non-responder). The non-responders were offered TURP.

*Genotyping Assay*s

Genomic DNA was extracted from peripheral blood by QIAamp DNA mini kit (QIAGEN, Germany). All patients and controls were genotyped for VDR (Fok-I, Taq-I and Bsm-I) by PCR-RFLP method using a programmed thermal cycler Master cycler gradient (Eppendorf, USA) as described earlier (Bid et al., 2005). Gels were visualized under ultraviolet light by using software in Biovis Gel Software, version-4 (Expert vision, Mumbai). Primers were synthesized commercially from (Gibco BRL Berlin Germany). All the restriction enzymes and the 100 bp DNA ladder were purchased from MBI-Fermentas, USA. To improve the genotyping quality and substantiation, 30% of samples were regenotyped by other laboratory personnel and results were reproducible with no discrepancy recorded in genotyping.

Statistical Analysis

The sample size was calculated using Quanto1.1 program (available at http://hydra.usc.edu/gxe). Allele frequencies, genotype frequencies and carriage rates of the alleles in all the groups were compared using a 2x2 contingency table by Fisher's exact test. The Hardy-Weinberg equilibrium at individual loci was accessed by χ^2 statistics using the programme SPSS software, version-11.5 (Chicago, IL). All p values were two sided and differences were considered statistically significant for $P<0.05$. Odds ratio (OR) at 95% CI was determined to describe the strength of association by Logistic Regression Model. To examine whether the genotype frequencies were in Hardy-Weinberg Equilibrium, Good- ness of fit χ^2 test was used.

Results and Discussion

The mean age of patient was 64.4+9.1 years. The observed Fok I, Bsm I, and Taq I genotype frequencies were in accordance with the Hardy-Weinberg equilibrium in both patients and controls (data not shown). Comparison of the frequency of genotype, allele and carriage rate of all the three VDR loci between patients and controls has been shown in Table 2. Statistical analyses of the genotype prevalence showed that significant differences in the Taq-I and Bsm-I genotype were observed (p=0.022 and 0.033). In allelic frequencies, significant differences in the Fok-I and Taq-I allelic prevalence were observed but lack of association was observed in carriage rate of patients and controls. In case of carriage rate also lack of association were observed between patients and control in all the three loci of VDR (Table 2). The distribution of all combined VDR genotypes in BPH patients and control group are shown in Table 3.

When we divided the subjects into responder and nonresponder groups as described above, significant genotypic difference was found with only in case of Taq-I (p=0.001) and no association were found at other VDR locus (Table 4). When compared between responder and non-responder patients, significant differences were also observed in the prevalence carriage rate of Fok- I, Taq-I and Bsm-I (p=0.000, 0.004 and 0.00) alleles.

Candidate gene polymorphism association studies are widely employed tool to identify genetic factors conferring susceptibility to complex disorders like BPH (Konwar et

Table 1. World-Wide Study of Genetic Variants of VDR and Association with Risk of BPH

SL. No	VDR Site	BPH Patients	Mean Age	Association	Country	Reference
1	BsmI, ApaI & Taq I	209	70.4 ± 9.4	S	Japan	Zamuda et al., 2000
2	BsmI, ApaI & Taq I	44	NA	NS	Bangkok	Chaimuangraj et al., 2006
3	TaqI	83	NA	NS	Japan	Hamasakia et al,. 2002
4	Taq I	98	66.4 ± 7.3	NS	Netherland	Bousema et al., 2000
5	Fok-I	189	68.6 ± 8.5	NS	Taiwan	Huang et al., 2006

* S-Significant, NS-Non significant

Table 2. Frequency Distribution, Allelic Frequencies and Carriage Rates of VDR Gene Polymorphisms in BPH Patients and Healthy Controls

VDR Gene	Controls, n (%), 160	Patients, n (%), 160	OR (95% CI) [P]
Fok-I			
FF	82 (51.25)	94 (58.0)	0.382(0.039-3.745)
Ff	77 (48.12)	63 (39.0)	0.273(0.028-2.687)
ff	1 (0.63)	3 (2.0)	1.0(ref) [0.210]
Taq-I			
TT	70 (43.75)	92 (57.5)	2.464(1.246-4.873)
Tt	60 (37.5)	52 (32.5)	1.625(0.798-3.310)
tt	30 (18.75)	16 (10.0)	10 (ref) [0.022]
Bsm-I			
BB	56 (35.0)	42 (26.25)	1.172(0.557-2.466)
Bb	79 (49.38)	102 (63.75)	2.017(1.009-4.034)
bb	25 (15.62)	16 (10.0)	10 (ref) [0.033]
Allelic Frequency			
Fok-I			
F	241 (75.31)	254 (79.37)	0.32 (0.234-0.458)
f	79 (24.69)	66 (20.63)	1.0 (ref) [0.000]
Taq-I			
T	200 (62.5)	236 (73.75)	1.686(1.204-2.360)
t	120 (37.5)	84 (26.25)	1.0 (ref) [0.012]
Bsm-I			
B	191 (59.69)	186 (58.12)	0.937(0.684-1.285)
b	129 (40.31)	134 (41.88)	1.0 (ref) [0.758]
Carriage Rate			
Fok-I			
F	159 (99.36)	157 (98.13)	1.167(0.786-1.732)
f	78 (48.75)	66 (20.63)	1.0 (ref) [0.318]
Taq-I			
T	130 (81.25)	144 (90)	1.466(0.988-2.174)
t	90 (56.25)	68 (42.5)	1.0 (ref) [0.081]
Bsm-I			
B	135 (84.38)	144 (90.0)	0.940(0.660-1.338)
b	104 (65.0)	118 (73.75)	1.0 (ref) [0.348]

al., 2008). But, most of the reported association studies are underpowered to detect the modest genetic effects underlying the genetic susceptibility of these common diseases (Rich et al., 2000).

There were contradictory reports of VDR polymorphism association with BPH. These could be due to false-positive studies or false negative studies. However, true variability in associations among different populations also could not be ignored (Lohmueller et al., 2003). Considering the heterogeneity of reports we took additional precaution for reconfirming genotyping data at several points to reduce false-positive/negative results. Our results demonstrate significant association of VDR (Taq-I and Bsm-I) with the risk for BPH (Table 2). These observations concur with previous reports in a Japanese population, where there was also an increased risk of BPH for these two loci (Habuchi et al., 2000). On the contrary, in Bangkok, Taiwan, Netherland and another Japanese studies, no association with the VDR (Taq-I and Bsm-I) genotype was reported (Table 1).

We also compared the three VDR genotypes between responder and nonresponder patient groups and significant genotypic difference was found in Taq-I variants of VDR (Table 4). The carriage rate was also significant in the entire three VDR loci between both the groups. This in our view is the first such study conducted to investigate

Table 3. Distribution of Combined VDR Genotypes in BPH Patients and Healthy Controls

Genotypes	Controls	Patients	P value	OR (95% CI)
FFBBTT	4	14	0.41	3.50 (0.18-69.34)
FFBBTt	6	8	0.85	1.33 (0.07-25.91)
FFBBtt	3	4	0.86	1.33 (0.06-31.12)
FFBbTT	18	32	0.69	1.78 (0.11-30.17)
FFBbTt	10	12	0.90	1.20 (0.07-21.72)
FFBbtt	5	4	0.89	0.80 (0.04-17.20)
FFbbTT	2	4	0.68	2.0 (0.08-51.59)
FFbbTt	2	3	0.81	1.5 (0.06-40.63)
FFbbtt	3	2	0.81	0.67 (0.03-18.06)
FfBBTT	10	8	0.89	0.80 (0.04-14.89)
FfBBtt	7	2	0.44	0.29 (0.01-6.91)
FfBbTT	13	18	0.82	1.38 (0.08-24.22)
FfBbTt	12	11	0.95	0.92 (0.05-16.49)
FfBbtt	8	9	0.94	1.13 (0.06-21.09)
FfbbTT	2	6	0.50	3.0 (0.12-73.64)
FfbbTt	5	3	0.75	0.60 (0.03-13.58)
ffBBTT	1	0	1.0	0.00
ffBBTt	0	1	1.0	0.00
ffBBtt	0	0	0.00	0.00
ffBbTT	0	1	1.0	0.00
ffbbTT	0	0	0.00	0.00
ffbbTt	0	0	0.00	0.00

Table 4. Comparison of Frequency Distribution, Allelic Frequencies and Carriage Rates of VDR Genotypes Between Responder and Non-Responder Group

VDR Gene	Responder, n =115	Non responder, n=33	OR (95% CI) [P]
Fok-I			
FF	70 (60.9)	18 (54.5)	0.382 (0.039-3.745)
Ff	44 (38.3)	12 (36.4)	0.091 (0.009-0. 955)
ff	1 (0.9)	3 (9.1)	1 (ref) [0.115]
Taq-I			
TT	65 (56.5)	20 (60.6)	0.192 (0.057-0.654)
Tt	45 (39.1)	5 (15.2)	0.069 (0.016-0.296)
tt	5 (4.3)	8 (24.2)	(ref) [0.001]
Bsm-I			
BB	31 (27.0)	9 (27.3)	1.742 (0.185-16.417)
Bb	78 (67.8)	23 (69.7)	1.769 (0.203-15.453)
bb	6 (5.2)	1 (3.0)	1.0 (ref) [0.875]
Allelic Frequency			
Fok-I			
F	185 (80.4)	48 (72.7)	0.75 (0.185-3.045)
f	45 (19.6)	18 (27.3)	1.0 (ref) [0.687]
Taq-I			
T	175 (76.1)	45 (68.2)	0.381 (0.073-1.991)
t	55 (23.9)	21 (31.8)	1.0 (ref) [0.253]
Bsm-I			
B	140 (60.9)	41 (62.1)	2.562 (0.860-7.633)
b	90 (39.1)	25 (37.9)	1.0 (ref) [0.091]
Carriage Rate			
Fok-I			
F	105 (91.30)	30(90.91)	0.857 (0.421-1.746)
f	45 (39.13)	15(45.45)	1.0(ref) [0.000]
Taq-I			
T	110 (95.65)	25(75.76)	0.590 (0.380-0.915)
t	50 (43.48)	13(39.39)	1.0 (ref) [0.004]
Bsm-I			
B	109 (94.78)	32 (99.97)	1.028 (0.563-1.874)
b	84 (70.04)	24 (72.73)	1.0 (ref) [0.00]

association of VDR polymorphism with response pattern of patients to standard BPH drug therapy.

The potential limitations of the current study are that the level of expression of VDR gene, level of vitamin D could not be acquired which would have further strengthened the findings of our study. Also, a combination of related genes like AR, steroid-metabolizing enzyme could have generated better and complete picture of susceptibility of BPH.

Our results established that Taq-I and Bsm-I VDR gene variant are associated with the susceptibility BPH in Indian population. VDR Taq-I genotype is significantly associated with the improvement of BPH patients with standard drug therapy. This first study from the India represents role of VDR gene polymorphisms in BPH of Indian population. Since VDR gene variants were suggested for race or ethnicity specific variability, it would assist in predicting the disease risk in India population and in narrowing down the reported variations.

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References

Adorini L, Penna G, Amuchastegui S (2007). Inhibition of prostate growth and inflammation by the vitamin D receptor agonist BXL-628 (elocalcitol). *J Steroid Biochem Mol Biol*, **103**, 689-93.

Bid HK, Mishra DK, Mittal RD (2005). Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population. *Asian Pac J Cancer Prev*, **6**, 147-52.

Blazer DG 3rd, Umbach DM, Bostick RM (2000). Vitamin D receptor polymorphisms and prostate cancer. *Mol Carcinogenesis*, **27**, 18-23.

Bousema TJ, Bussemakers MJG, van Houwelingen KP (2000). Polymorphisms in the vitamin D receptor gene and the androgen receptor gene and the risk of benign prostatic hyperplasia. *Eur Urol*, **37**, 234-8.

Crescioli C, Maggie M, Vannelli GB (2000). Effect of a vitamin D3 analogue on keratinocyte growth factor-induced cell proliferation in benign prostate hyperplasia. *J Clin Endocrinol Metab*, **85**, 2576-83.

Chaimuangraj S, Thammachoti R, Ongphiphadhanakul B (2006). Lack of association of VDR polymorphisms with Thai prostate cancer as compared with benign prostate hyperplasia and controls. *Asian Pac J Cancer Prev*, **7**, 136-9.

Feldman D, Malloy PJ, Gross C (2001). Vitamin D: biology, action and clinical implications. In: Marcus R, Feldman D and Kelsey J (Eds). Osteoporosis. Academic Press, San Diego.

Hammarsten J, Damber JE, Johnell O (2006). A low vitamin D level is an independent risk factor for the development of benign prostatic hyperplasia. *Urology*, **68**, 5.

Hamasakia T, Inatomia H, Katohb T (2002). Significance of vitamin D receptor gene polymorphism for risk and disease severity of prostate cancer and benign prostatic hyperplasia in Japanese. *Urol Int*, **68**, 226-31.

Habuchi T, Suzuki T, Sasaki R (2000). Association of vitamin D receptor gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. *Cancer Res*, **60**, 305-8.

Huang S, Huang C, Wu W (2006). Association of vitamin D receptor FokI polymorphism with prostate cancer risk, clinicopathological features and recurrence of prostate specific antigen after radical prostatectomy. *Int J Cancer*, **119**, 1902-7.

Krill D, DeFlavia P, Dhir R (2001). Expression patterns of vitamin D receptor in human Prostate. *J Cell Biochem*, **82**, 566-72.

Konety BR, Schwartz GG, Acierno JS (1996). The role of vitamin D in normal prostate growth and differentiation. *Cell Growth Differ*, **7**, 1563-70.

Konwar R, Chattopadhyay N, Bid HK (2008). Genetic polymorphism and pathogenesis of benign prostatic hyperplasia. *BJU Int*, **102**, 536-44.

Lohmueller KE, Pearce CL, Pike M (2003). Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet*, **33**, 177-82.

Risch NJ (2000). Searching for genetic determinants in the new millennium. *Nature*, **405**, 847-56.

Zamuda JM, Cauley JA, Ferrell RE (2000). Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev*, **22**, 203-17.