Vitamin D Receptor Genetic Polymorphisms Are Associated with PSA Level, Gleason Score and Prostate Cancer Risk in African-American Men

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Abstract. *Background/Aim: Several studies have revealed an association between single nucleotide polymorphisms (SNPs) in the VDR gene and prostate cancer (PCa) risk in European and Asian populations. To investigate whether VDR SNPs are associated with PCa risk in African-American (AA) men, nine VDR SNPs were analyzed in a case-control study. Materials and Methods: Multiple and binary logistic regression models were applied to analyze the clinical and genotypic data. Results: rs731236 and rs7975232 were significantly associated with PCa risk ($p<0.05$). In the analysis of clinical phenotypes, rs731236, rs1544410 and rs3782905 were strongly associated with high PSA level ($p<0.05$), whereas rs1544410 and rs2239185 showed a statistically significant association with high Gleason score ($p<0.05$). Haplotype analysis revealed several VDR haplotypes associated with PCa risk. Additionally, a trend existed, where as the number of risk alleles increased in the haplotype, the greater was the association with risk (p-trend=0.01). Conclusion: These results suggest that the VDR SNPs may be associated with PCa risk and other clinical phenotypes of PCa in AA men.*

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Prostate cancer (PCa) is one of the most commonly diagnosed forms of cancer among men in the developed world (1, 2). In 2013, it was estimated that 238,590 men were diagnosed and 29,720 men died of PCa in the United States; and in 2014, there will be an estimated 1,665,540 new diagnosed cancer cases and 585,720 cancer deaths in the United States (3, 4). There are well-established risk-factors, such as genetic predisposition, age, ethnicity, family history, diet and environmental factors that contribute to the etiology of PCa (5). Additionally, there is a discrepancy in the risk of developing PCa among developed and underdeveloped countries and among ethnic groups. For example, the incidence and mortality rate of PCa in African-American (AA) is similar to men from the Caribbean and South America with West African ancestry. In the United States, though, there is a two-fold increased PCa incidence when comparing AA men with their Caucasian counterparts. Still, although epidemiological studies have shown that the incidence of PCa is lower in Asians, the occurrence of this disease has rapidly increased among Chinese men (6, 7). Such disproportionate difference in the prognosis and mortality of PCa among ethnic groups remains unclear.

Prostate cell division is influenced by two steroid hormones: testosterone and vitamin D. The action of these hormones is mediated by their respective receptors: androgen receptor (AR) and vitamin D receptor (VDR). The hormonal active form of vitamin D 1, 25-dihydroxyvitamin D inhibits cancer cell growth angiogenesis and metastasis (8-11) and Vitamin D is one of the hormones that have been known to be associated with a lower risk of several types of cancer, including PCa (12-14).

Vitamin D receptor, a nuclear transcription regulating factor that functions in signaling the synthesis of proteins

involved in bone mineral homeostasis and cell-cycle regulation, mediates the action of vitamin D (14). The human *VDR* gene is found on chromosome 12q13.11 and is made up of 14 exons spanning approximately 75 kb (15, 16). It is highly polymorphic with at least 618 reported variants, most of which are either not detectable or at a low frequency in the general population, according to the dbSNP database (17). Previous studies have primarily focused on four common variants hypothesized to influence the expression and/or function of the VDR protein. Single nucleotide polymorphisms (SNPs) in the *VDR* gene, Bsm1 and FokI, have been inconsistently associated with breast cancer risk (18). Similarly identified SNPs in the *VDR* gene from population-based studies have also been associated with PCa risk (19-21, 21). Two SNPs, *Apal* and *Bsml*, are located in intron 8, while *Taq1* is located in exon 9. Additionally, *Fok1* is located in exon 2 and leads to a C/T substitution; its absence results in a truncated protein with greater luciferase activity (22). A G/A polymorphism in the promoter region of the *VDR* gene has also been shown to interact with the caudal related homeodomain transcription factor (CDX2); the common *CDX2* G allele has 70% of the transcriptional activity compared with the A allele (23).

Genetic polymorphisms and PCa association have been extensively studied generating mixed results (24-28), while some failed to conclude any positive associations of *VDR* polymorphisms and PCa (29-32). Because few studies have been performed in populations of African descent, we therefore embarked on a study to determine if DNA variation in *VDR* was associated with increased risk of PCa in AA men, as well as other clinical phenotypes, such as Gleason score. To test our hypothesis that *VDR* genotypes and haplotypes were indeed associated with PCa risk, we conducted a case-control study to determine the association of nine selected *VDR* SNPs with prostate specific antigen (PSA), Gleason Score and PCa risk.

Materials and Methods

Study population. This case-control study is comprised of 2 cohorts: the AA Sporadic PCa Study (AAPCA) conducted at the National Human Genome Center (NHGC) at the Howard University and the Vitamin D and PCa Risk in AA Men Study. From these two studies, we selected 446 AA men aged 35 to 93 years from the Washington DC area with histologically-diagnosed adenocarcinoma of the prostate, PSA of >4.0 ng/ml and a positive digital rectal examination (DRE). Ethnicity-matched controls (n=379) with PSA levels <4.0 ng/ml, normal DRE and with no history of PCa among first-degree relatives were also recruited. Participants were recruited from the Division of Urology at the Howard University Hospital (HUH) and/or from ongoing free PCa screening program at the Howard University Cancer Center (HUCC). Blood samples were drawn from each participant and clinical characteristics, such as Gleason score and PSA level were obtained from the HUH pathology records. The Howard University Institutional Review Board (IRB) approved the study protocol (IRB-02-MED-42; IRB-11-MED-22) and written informed consent was obtained from all study subjects.

Table I. *Characteristics of study subjects*.

Characteristics	Controls	Cases	*p*-Value
Number of subjects	379	446	
Mean Age (±SD)	57.7 (±10.8)	65.47 (±9.18)	0.0001
Mean PSA level	2.52 (±2.46)	97.19 (±421.73)	0.0002
Average Gleason score		7.8 (±0.63)	

Plus-minus values are means±SD. Prostate -specific antigen (PSA) levels were obtained at the time of diagnosis for cases and at the time of study enrollment for the controls.

SNPs selection and genotyping. To explore *VDR* SNPs with likely association to the biology of PCa in AA men, SNPs in the National Center for Biotechnology Information (17) were prioritized using a SNP prioritization algorithm developed at the NHGC. First, based on power and sample size calculations, SNPs with minor allele frequencies (MAF) greater than 10% were chosen. Then, MAFs were compared in Caucasian and Yoruba populations using chi square analysis of significance to identify SNPs with differential allele frequencies (Δ MAF). Greater consideration was given to SNPs found in exonic region that resulted in non-synonymous (missense) amino acid changes. Also, selected SNPs were in splice junctions, promoter and 3' untranslated regions (UTRs). At the end, the following nine SNPs were selected for this study: rs731236 (*TaqI*, exon 9), rs7975232 (*ApaI*, intron 8), rs1544410 (*BsmI*, intron 8), rs3782905 (IVS4+6584), rs2239185 (59256C>T), rs2289179 (46049A>G), rs10783218 (31072C>T), rs4516035 (3989A>G) and rs2853563 (68077G>A).

DNA extraction and genotyping. Genomic DNA extraction was performed on whole blood using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Genomic DNA from each subject was used for genotyping the 9 selected SNPs. Genotyping was performed by using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). Custom genotyping assays were designed for each SNP and optimized. Reactions were run in parallel with the non-template control (master mix and molecular grade water). For quality control, subjects were genotyped in duplicate and the overall concordance rate was 99.9%. The ABI 7900HT Sequence Detection System (SDS) software (version 2.0) was used to analyze real-time and end-point fluorescence and allelic discrimination (Applied Biosystems).

Statistical analyses. The statistical analyses were performed using the SPSS (version 20.0) and SNPStats (http://bioinfo.iconcologia.net/SNPstats) (33). Allele frequencies in controls were tested for Hardy-Weinberg equilibrium using chi square analysis or Fisher's exact test when appropriate. The association of disease status and other clinical phenotypes with genotype and haplotype was analyzed by binary and multivariate logistic regression. For each SNP genotype, tests using the genotypic, dominant, recessive and log additive genetic models were performed. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95%

Table II. *Characterization of VDR SNPs and summary of their association with PSA level, Gleason Score (GS) and PCa risk.*

SNPs	Other name	Location	Ancestral allele	YRI Allele frequency	CEU Allele frequency	High PSA risk allele	PCa risk allele	High GS risk allele
rs4516035		5'UTR	T	0.992	0.542			
rs10783218		Intron 1	C	0.686	0.983			
rs2289179		Intron 2	A	0.708	0.535		C	
rs3782905		Intron 2	C	0.783	0.702	C	C	
rs2239185		Intron 7	C	0.458	0.417			A
rs1544410	BsmI	Intron 8	G	0.721	0.562	G		T
rs7975232	ApaI	Intron 8	C	0.372	0.429		C	
rs731236	TaqI	Ile352Ile (Ex 9)	T	0.712	0.562	T	T	
rs2853563		3'UTR	G	0.823	0.965			

* YRI, Yoruban in Ibadan and Nigeria; CEU, Northern and Western European ancestry.

confidence intervals (95% CI), adjusted for age for the association between individual SNP and PCa risk. Two-sided *p*-values of ≤0.05 were considered as statistically significant. Using the Bonferroni test, adjustment was made for multiple comparisons testing. To test the combinatorial effects of the SNPs and haplotypes, frequencies were estimated using the implementation of the EM algorithm coded into the haplostats package (25).

Results

In this study, 446 cases and 379 controls were investigated to explore the association of *VDR* SNPs and PCa risk. The characteristics of PCa patients and control subjects are shown in Table I. The respective mean age of patients with PCa and control subjects were 65.5±9.18 and 57.7±10.8 years, respectively. A Student's *t*-test reveals significant differences in age between the cases and controls ($p<0.0001$). The mean Gleason score of the PCa was 7.8±0.63. The mean PSA levels were 2.5 ng/ml and 97.2 ng/ml for the controls and cases, respectively ($p=0.0002$). All nine SNPs rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*), rs3782905, rs2239185, rs2289179, rs10783218, rs4516035 and rs2853563 were in Hardy Weinberg equilibrium ($p>0.05$). Six of the 9 SNPs were found to be associated with PSA level, PCa risk and/or Gleason score (Table II). SNPs rs731236 and rs3782905 were associated with PSA level and PCa risk. SNP rs1544410 was associated with PSA level and Gleason score, while rs2239185 was associated only with Gleason score. Two SNPs, rs2289179 and rs7975232, were associated only with PCa risk.

The SNPs associated with PSA level are listed in Table III. SNP rs731236 was found to be associated with PSA risk in AA men even after adjusting for age using the codominant ($p=0.021$), dominant (OR=0.50, 95% CI: 0.29-0.87; $p=0.014$), recessive (OR=0.41, 95% CI: 0.17-0.95; p=0.045) and the log additive models (OR=0.56, 95% CI: 0.37-0.84; $p=0.005$). SNP rs1544410 was also found to be associated with PSA in AA men after adjusting for age using the recessive (OR=0.42, 95% CI: 0.18-0.93; $p=0.04$) and the log additive models (OR=0.63, 95% CI: 0.42-0.95; $p=0.027$). Finally, SNP rs3782905 was also found to be significantly associated with PSA risk in our populations after adjusting for age using the codominant (OR=0.60, 95% CI: 0.15-2.41; $p=0.045$), dominant (OR=0.49, 95% CI: 0.28-0.86; $p=0.014$), over dominant (OR=0.50, 95% CI: 0.28-0.88; $p=0.017$) and the log additive models (OR=0.59, 95% CI: 0.37-0.94; $p=0.028$). After multiple comparisons testing, rs2289179 lost its significant association under the over dominant model.

Additionally, the association between *VDR* SNPs and PCa risk was also assessed (Table IV). SNP rs731236, was found to be significantly associated with PCa risk in AA men after adjusting for age using the codominant (OR=0.68, 95% CI: 0.45-1.01; $p=0.039$ and OR=0.49, 95% CI: 0.26-0.95; $p=0.39$), dominant (OR=0.63, 95% CI: 0.43-0.92; $p=0.018$) and the log additive models (OR=0.69, 95% CI: 0.52-0.92; $p=0.011$). SNP rs7975232 was also found to be significantly associated with PCa risk using the recessive model (OR= 1.85, 95% CI: 1.01-3.39; $p=0.042$). Furthermore, rs3782905 was found to be marginally significant using the dominant model (OR= 0.72, 95% CI: 0.50-1.02; $p=0.06$), but lost its significance after adjusting for age. Finally, rs2239185 completely lost its significant association when adjusted for age, although the recessive model showed marginal significance after adjusting for age (OR= 1.70, 95% CI: 0.97-3.00; $p=0.061$).

The association between *VDR* SNPs and Gleason score was also determined (Table V). High and low Gleason scores were defined as less advanced "if a case's Gleason score is <7" and more advanced "if their Gleason score is >7". SNP rs1544410 was found to be significantly associated with PCa and high Gleason score risk when adjusted for age under the codominant (OR=1.65, 95% CI: 0.59-4.59; $p=0.022$ and OR=1.92, 95% CI: 0.24-15.00; $p=0.022$) and the recessive models (OR=1.61, 95% CI: 0.21-12.18; $p=0.0071$). SNP rs2239185 was found to be significantly associated with high

Table III. *VDR SNPs association with prostate-specific antigen (PSA) level.*

rs731236							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	T/T	27 (38.6%)	157 (57.1%)	1	0.013		0.021
	C/T	33 (47.1%)	99 (36.0%)	0.52 (0.29-0.91)		0.57(0.32-1.01)	
	C/C	10 (14.3%)	19 (6.9%)	0.33 (0.14- 0.78)		0.31 (0.13-0.77)	
Dominant	T/T	27 (38.6%)	157 (57.1%)	1	0.005	0.50-(0.29-0.87)	0.014
	C/T-C/C	43 (61.4%)	118 (42.9%)	0.47 (0.28-0.81)			
Recessive	T/T-C/T	60 (85.7%)	256 (93.1%)	1	0.062	0.41 (0.17-0.95)	0.045
	C/C	10 (14.3%)	19 (6.9%)	0.45 (0.20-1.01)			
Over dominant	T/T-C/C	37 (52.9%)	176 (64.0%)	1	0.090	0.69 (0.40-1.20)	0.190
	C/T	33 (47.1%)	99 (36.0%)	0.63 (0.37-1.07)			
Log additive	–	–	–	0.55 (0.37-0.82)	0.003	0.56 (0.37-0.84)	0.005
rs1544410							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	G/G	27 (38.0%)	139 (50.0%)	1.00	0.079	1.00	0.069
	A/G	33 (46.5%)	117 (42.1%)	0.69 (0.39-1.21)		0.73 (0.41-1.30)	
	A/A	11 (15.5%)	22 (7.9%)	0.39 (0.17-0.89)		0.35 (0.15-0.84)	
Dominant	G/G	27 (38.0%)	139 (50.0%)	1.00	0.070	1.00	0.097
	A/G-A/A	44 (62.0%)	139 (50.0%)	0.61 (0.36-1.05)		0.63 (0.37-1.09)	
Recessive	G/G-A/G	60 (84.5%)	256 (92.1%)	1.00	0.065	1.00	0.040
	A/A	11 (15.5%)	22 (7.9%)	0.47 (0.22-1.02)		0.42 (0.18-0.93)	
Over dominant	G/G-A/A	38 (53.5%)	161 (57.9%)	1.00	0.510	1.00	0.690
	A/G	33 (46.5%)	117 (42.1%)	0.84 (0.50-1.41)		0.90 (0.52-1.54)	
Log additive	–	–	–	0.64 (0.43-0.95)	0.026	0.63 (0.42-0.95)	0.027
rs2289179							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	T/T	24 (35.3%)	128 (45.7%)	1.00	0.16	1.00	0.25
	C/T	37 (54.4%)	116 (41.4%)	0.59 (0.33-1.04)		0.61 (0.34-1.10)	
	C/C	7 (10.3%)	36 (12.9%)	0.96 (0.38-2.42)		0.81 (0.31-2.07)	
Dominant	T/T	24 (35.3%)	128 (45.7%)	1.00	0.12	1.00	0.12
	C/T-C/C	44 (64.7%)	152 (54.3%)	0.65 (0.37-1.12)		0.64 (0.36-1.13)	
Recessive	T/T-C/T	61 (89.7%)	244 (87.1%)	1.00	0.56	1.00	0.91
	C/C	7 (10.3%)	36 (12.9%)	1.29 (0.55-3.03)		1.05 (0.43-2.54)	
Over dominant	T/T-C/C	31 (45.6%)	164 (58.6%)	1.00	0.05	1.00	0.11
	C/T	37 (54.4%)	116 (41.4%)	0.59 (0.35-1.01)		0.64 (0.37-1.10)	
Log additive	–	–	–	0.85 (0.58-1.24)	0.4	0.80 (0.53-1.19)	0.27
rs3782905							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	C/C	30 (45.5%)	158 (63.5%)	1.00	0.028	1.00	0.045
	C/T	33 (50.0%)	81 (32.5%)	0.47 (0.27-0.82)		0.48 (0.27-0.86)	
	T/T	3 (4.5%)	10 (4.0%)	0.63 (0.16-2.44)		0.60 (0.15-2.41)	
Dominant	C/C	30 (45.5%)	158 (63.5%)	1.00	0.008	1.00	0.014
	C/T-T/T	36 (54.5%)	91 (36.5%)	0.48 (0.28-0.83)		0.49 (0.28-0.86)	
Recessive	C/C-C/T	63 (95.5%)	239 (96.0%)	1.00	0.850	1.00	0.780
	T/T	3 (4.5%)	10 (4.0%)	0.88 (0.23-3.29)		0.82 (0.21-3.22)	
Over dominant	C/C-T/T	33 (50.0%)	168 (67.5%)	1.00	0.009	1.00	0.017
	C/T	33 (50.0%)	81 (32.5%)	0.48 (0.28-0.84)		0.50 (0.28-0.88)	
Log additive	–	–	–	0.59 (0.37-0.92)	0.022	0.59 (0.37-0.94)	0.028

*OR (95% CI): odd ratio and 95% confidence interval of SNP response to PSA when adjusted by age; **p-value, adjusted *p*-value by age.

Table IV. *SNPs association with prostate cancer risk*.

rs731236							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	T/T	105 (41.8%)	170 (55.6%)	1.00	0.004	1.00	0.039
	C/T	115 (45.8%)	111 (36.3%)	0.60 (0.42-0.85)		0.68 (0.45-1.01)	
	C/C	31 (12.3%)	25 (8.2%)	0.50 (0.28-0.89)		0.49 (0.26-0.95)	
Dominant	T/T	105 (41.8%)	170 (55.6%)	1.00	0.0012	1.00	0.018
	C/T-C/C	146 (58.2%)	136 (44.4%)	0.58 (0.41-0.81)		0.63 (0.43-0.92)	
Recessive	T/T-C/T	220 (87.7%)	281 (91.8%)	1.00	0.100	1.00	0.095
	C/C	31 (12.3%)	25 (8.2%)	0.63 (0.36-1.10)		0.59 (0.32-1.10)	
Over dominant	T/T-C/C	136 (54.2%)	195 (63.7%)	1.00	0.022	1.00	0.170
	C/T	115 (45.8%)	111 (36.3%)	0.67 (0.48-0.95)		0.76 (0.52-1.12)	
Log additive	–	–	–	0.66 (0.51-0.86)	0.001	0.69 (0.52-0.92)	0.011
rs7975232							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	A/A	125 (49.2%)	133 (42.9%)	1.00	0.023	1.00	0.12
	A/C	107 (42.1%)	127 (41.0%)	1.12 (0.78-1.59)		0.97 (0.65-1.45)	
	C/C	22 (8.7%)	50 (16.1%)	2.14 (1.22-3.73)		1.83 (0.97-3.45)	
Dominant	A/A	125 (49.2%)	133 (42.9%)	1.00	0.130	1.00	0.58
	A/C-C/C	129 (50.8%)	177 (57.1%)	1.29 (0.92-1.80)		1.11 (0.76-1.63)	
Recessive	A/A-A/C	232 (91.3%)	260 (83.9%)	1.00	0.007	1.00	0.04
	C/C	22 (8.7%)	50 (16.1%)	2.03 (1.19-3.45)		1.85 (1.01-3.39)	
Over dominant	A/A-C/C	147 (57.9%)	183 (59.0%)	1.00	0.780	1.00	0.44
	A/C	107 (42.1%)	127 (41.0%)	0.95 (0.68-1.33)		0.86 (0.59-1.26)	
Log additive	–	–	–	1.34 (1.05-1.71)	0.018	1.21 (0.92-1.60)	0.17
rs2239185							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	T/T	83 (35.6%)	90 (30.9%)	1.00	0.05	1.00	0.17
	C/T	123 (52.8%)	145 (49.8%)	1.09 (0.74-1.59)		1.02 (0.66-1.59)	
	C/C	27 (11.6%)	56 (19.2%)	1.91 (1.11-3.31)		1.73 (0.93-3.23)	
Dominant	T/T	83 (35.6%)	90 (30.9%)	1.00	0.26	1.00	0.51
	C/T-C/C	150 (64.4%)	201 (69.1%)	1.24 (0.86-1.78)		1.15 (0.76-1.75)	
Recessive	T/T-C/T	206 (88.4%)	235 (80.8%)	1.00	0.01	1.00	0.06
	C/C	27 (11.6%)	56 (19.2%)	1.82 (1.11-2.99)		1.70 (0.97-3.00)	
Over dominant	T/T-C/C	110 (47.2%)	146 (50.2%)	1.00	0.50	1.00	0.47
	C/T	123 (52.8%)	145 (49.8%)	0.89 (0.63-1.25)		0.87 (0.58-1.28)	
Log additive	–	–	–	1.31 (1.01-1.70)	0.03	1.24 (0.93-1.67)	0.14
rs3782905							
Model	Genotype	Controls	Cases	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	C/C	117 (51.1%)	163 (59.3%)	1.00	0.18	1.00	0.64
	C/T	98 (42.8%)	97 (35.3%)	0.71 (0.49-1.03)		0.82 (0.54-1.24)	
	T/T	14 (6.1%)	15 (5.5%)	0.77 (0.36-1.65)		0.97 (0.40-2.35)	
Dominant	C/C	117 (51.1%)	163 (59.3%)	1.00	0.066	1.00	0.38
	C/T-T/T	112 (48.9%)	112 (40.7%)	0.72 (0.50-1.02)		0.84 (0.56-1.25)	
Recessive	C/C-C/T	215 (93.9%)	260 (94.5%)	1.00	0.75	1.00	0.9
	T/T	14 (6.1%)	15 (5.5%)	0.89 (0.42-1.88)		1.06 (0.45-2.51)	
Over dominant	C/C-T/T	131 (57.2%)	178 (64.7%)	1.00	0.084	1.00	0.34
	C/T	98 (42.8%)	97 (35.3%)	0.73 (0.51-1.04)		0.82 (0.55-1.23)	
Log additive	–	–	–	0.79 (0.59-1.05)	0.1	0.89 (0.64-1.24)	0.5

*OR (95% CI), odd ratio and 95% confidence interval of SNP response to status when adjusted by age; **p-value, adjusted *p*-value by age.

Table V. *SNPs association with Gleason score (GS) in prostate cancer cases.*

				rs1544410			
Model	Genotype	Low GS	High GS	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	G/G	62 (53.0%)	44 (51.2%)	1.00	0.01	1.00	0.022
	A/G	50 (42.7%)	28 (32.6%)	0.79 (0.43-1.44)		1.65 (0.59-4.59)	
	A/A	5 (4.3%)	14 (16.3%)	3.95 (1.32-11.76)		1.92 (0.24-15.04)	
Dominant	G/G	62 (53.0%)	44 (51.2%)	1.00	0.80	1.00	0.680
	A/G-A/A	55 (47.0%)	42 (48.8%)	1.08 (0.62-1.88)		1.69 (0.64-4.46)	
Recessive	G/G-A/G	112 (95.7%)	72 (83.7%)	1.00	0.003	1.00	0.007
	A/A	5 (4.3%)	14 (16.3%)	4.36 (1.50-12.61)		1.61 (0.21-12.18)	
Over dominant	G/G-A/A	67 (57.3%)	58 (67.4%)	1.00	0.14	1.00	0.21
	A/G	50 (42.7%)	28 (32.6%)	0.65 (0.36-1.16)		1.56 (0.57-4.26)	
Log additive	–	–	–	1.38 (0.90-2.10)	0.14	1.51 (0.69-3.33)	0.14
				rs2239185			
Model	Genotype	Low GS	High GS	OR (95% CI)	*p*-Value	*OR (95% CI)	**p*-Value
Codominant	T/T	28 (26.2%)	61 (71.8%)	1.00	0.026	1.00	0.030
	C/T	65 (60.8%)	24 (28.2%)	0.60 (0.33-1.10)		0.40 (0.14-1.17)	
	C/C	14 (13.1%)	0 (0.0%)	0.00 (0.00-NA)		0.00 (0.00-NA)	
Dominant	T/T	28 (26.2%)	61 (71.8%)	1.00	0.048	1.00	0.062
	C/T-C/C	79 (73.8%)	24 (28.2%)	0.55 (0.30-1.00)		0.37 (0.13-1.04)	
Recessive	T/T-C/T	93 (86.9%)	85 (100%)	1.00	0.034	1.00	0.032
	C/C	14 (13.1%)	0 (0.0%)	0.00 (0.00-NA)		0.00 (0.00-NA)	
Over dominant	T/T-C/C	42 (39.2%)	61 (71.8%)	1.00	0.14	1.00	0.170
	C/T	65 (60.8%)	24 (28.2%)	0.63 (0.35-1.16)		0.44 (0.15-1.26)	
Log additive	–	–	–	0.52 (0.30-0.92)	0.022	0.36 (0.13-0.98)	0.028

*OR (95% CI), odd ratio and 95% confidence interval when adjusted by age; **p-value, adjusted *p*-value by age; NA, not applicaple

Table VI. *Haplotype frequencies of PCa risk alleles and association with PCa risk.*

Haplotype	rs731236	rs7975232	rs2339185	rs3782905	# Risk alleles	Control(%)	Cases(%)	OR (95% CI)	*p*
1	C	A	T	T	0	85 (0.146)	59 (0.102)	1.00	
2	C	A	T	C	1	71 (0.121)	71(0.122)	0.69 (0.43-1.11)	0.08
3	T	A	T	T	1	39 (0.068)	32 (0.056)	1.18 (0.67-2.09)	0.33
4	T	A	T	C	2	131 (0.224)	124 (0.212)	1.36 (0.90-2.06)	0.09
5	T	A	C	C	3	44 (0.075)	54 (0.093)	1.77 (1.05-2.97)	0.02
6	T	C	C	C	4	116 (0.198)	147(0.252)	1.82 (1.21-2.76)	0.00
								p-trend	0.01

OR (95% CI): odds ratio and 95% confidence interval.

Gleason score under the codominant (OR=0.40, 95% CI: 0.14-1.17; p=0.03), recessive p=0.032) and log additive models (OR=0.36, 95% CI: 0.13-0.98; p=0.028). Polymorphism rs2239185 became marginally significant (OR=0.37, 95% CI: 0.13-1.04; p=0.062) with high Gleason score under the dominant model when adjusted for age. Notably, there was an inverse relationship between Gleason score risk alleles and high PSA level, as well as PCa risk alleles.

A haplotype analysis of *VDR* SNPs revealed several associated haplotypes with PCa risk. Specifically, haplotype analyses of SNPs associated with PCa risk and those not associated with PCa risk were undertaken separately. The haplotype analyses made up of PCa associated risk alleles showed an additive effect with increasing risk alleles (p-trend<0.001) (Table VI). In particular, the greater the number of risk alleles in the haplotype, the greater the risk.

Table VII. *Haplotype frequencies and association with non-associated PCa risk alleles.*

Haplotype	rs	rs	rs	rs	rs	Control	Cases	OR	*p*	*OR	*p-Value
	1544410	2289179	2853563	4516031	10783218	(%)	(%)	(95% CI)	(95% CI)		
1	G	T	G	C	C	186(0.32)	196(0.34)	1	–	1	–
2	A	C	G	C	C	73(0.13)	55(0.09)	1.41 (0.81-2.44)	0.23	1.34 (0.74-2.45)	0.34
3	G	T	A	C	C	73(0.13)	56(0.09)	1.36 (0.80-2.31)	0.26	1.29 (0.65-2.54)	0.47
4	G	C	G	C	C	56(0.09)	57(0.09)	1.08 (0.64-1.80)	0.78	1.17 (0.61-2.22)	0.64
5	G	T	G	C	T	45(0.08)	44(0.07)	1.07 (0.58-1.97)	0.83	0.97 (0.47-2.01)	0.94
6	A	T	G	C	C	42(0.07)	36(0.06)	1.25 (0.69-2.25)	0.46	1.28 (0.65-2.54)	0.47

*OR (95% CI), odd ratio and 95% confidence interval when adjusted by age; **p-value, adjusted p-value by age.

Compared to the reference haplotype made up of the non-risk alleles, haplotype 6, made up of 4 risk alleles, showed an odds ratio of 1.82 (95% CI: 1.21-2.76; p=0.008). The haplotype with 3 risk alleles was also associated with PCa risk (OR=1.77, 95% CI: 1.05-2.97; p=0.02). SNPs that were not associated with PCa also underwent haplotype analysis. All of the haplotypes remained not significantly associated with PCa risk, thus indicating no additive or multiplicative effect (Table VII).

Discussion

In the present study, we investigated the association between nine genetic variants in VDR (rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*), rs2289179, rs2239185, rs2853563, rs4516035, rs10783218, and rs3782905 (IVS4+6584)) and PCa risk in AA population. Our results suggest that *VDR* SNPs might be associated with risk and severity of PCa.

Several studies and meta-analyses have documented the association between genetic variants in VDR and PCa risk (26-31). While most studies have focused mainly on *BsmI, TaqI, FokI, ApaI* and the *poly-A* microsatellite, all have shown an association with risk of PCa except *FokI* variants. Oakley-Girvan and colleagues (34) observed an increased risk associated with homozygosity for the F allele at the FokI site that was stronger in AA cases with advanced disease than in those with localized disease (34). In our study, we found an association between *TaqI*, *ApaI* and rs2239185 with PCa risk in our population, while Oakley-Girvan *et al*. (34) did not find an association among *BsmI, ApaI,* or *TaqI* and *poly-A* microsatellite with PCa risk in the family or case-control data (34).

Our important finding and other studies identified the association of *VDR* with PCa Gleason score (35, 36). Gleason score is accepted as a valid and reliable measure of assessing the aggressiveness and extent of PCa. Also, Gleason score was found to have a high predictive accuracy for biochemical recurrence compared to TNM staging system in a study of advanced-PCa patients (37). In our study, we found an association between *BsmI* and rs2239185 genotypes and high Gleason score. However, the complete pathological staging was not available in the majority of our cases and this is potentially cofounding the lack of correlation with stage in our study.

It has been reported that *BsmI* and *TaqI* sites are very tightly linked in Whites (38, 39) and are somewhat less linked in AA (40). Our haplotype analysis for SNPs in the *VDR* gene revealed that rs2239185 is in linkage disequilibrium with rs2289179 and *ApaI*, while *TaqI* and *ApaI* are in linkage disequilibrium and are located 80 bps apart. Furthermore, rs52853563 is in linkage disequilibrium with *BsmI* and with rs4516035, rs10783218 and rs3782905. It has been reported that the *ApaI, TaqI, BsmI* SNPs are all located in one block, which is thought to contain other variants at 3'-untranslated region of the *VDR* gene that may affect *VDR* expression by altering the mRNA stability and thus have effect on the vitamin D metabolism and activity (38, 41). However, the *TaqI* polymorphism is not functional but is in linkage disequilibrium with a *poly-A* microsatellite repeat in the 3' untranslated region (UTR) that is thought to be important in post-transcriptional control gene expression. The SNP rs3782905 is in the DNA binding domain responsible for interaction with vitamin D response elements (VDREs) in target genes (42).

In general, SNPs tend to be inherited in groups and these groups of SNPs and their various possible combinations may have a significant association with the disease phenotype. Haplotype analysis is likely to continue to play a key role in genetic epidemiology studies (43) because it effectively captures both the joint marker correlations and the evolutionary history.

SNPs in the *VDR* gene may be the candidates for predisposition to PCa given their function and association with other cancers (6, 44). VDR is an intracellular hormone receptor that specifically binds 1, 25(OH) 2D3 and mediates its effects (45, 46). The VDR is the mediator of all genomic actions of vitamins D3 and its analogs. It belongs to a family of ligand induced transcription factors, nuclear receptors

(NRs). Vitamin D3 is the main regulator of calcium homeostasis and is critical in bone formation. It is also involved in controlling cellular growth, differentiation and apoptosis, which makes synthetic vitamin D3 analogues interesting for therapy of such diseases as cancer and psoriasis (47). VDR acts primarily as a heterodimer with the retinoid X receptor (RXR) on VDREs. It interacts with the transcription machinery and nuclear receptor co-activators or co-repressors to regulate target gene activity. As the VDR/RXR heterodimer also represses transcription in a ligand-dependent manner through negative VDRE (nVDRE), a number of co-repressor proteins, such as NCoR and ALIEN may also be recruited to the surface of the receptor. They too function as platforms but serve to recruit enzymes, such as histone deacetylases. WINAC association with VDR facilitates targeting of a putative co-repressor complex to the nVDRE (48-52). One could speculate that high expression of *VDR* gene in cancer will lead to a decreased apoptosis activity (12). Therefore, changes in VDR may also have direct effects on vitamin D signaling and downstream effects on gene expression, which will in turn control cell proliferation. Remarkably SNPs will cause a dysfunctional receptor that may hinder its dimerization with RXR, thus failing to regulate target gene activity (cellular and growth apoptosis) leading to cancer.

Conclusion

We evaluated the relationship between nine SNPs of the *VDR* gene and the risk of prostate cancer. To our knowledge, we are the first to report the association between *VDR* rs2239185 SNP with high Gleason score. Our sample size was relatively small; therefore, if our present finding among AA at *TaqI*, *ApaI* and rs2239185 sites can be confirmed in a larger study, the population-specific significance of these *VDR* genetic variants in PCa etiology will be an important area for additional research and may result in screening and treatments that reduce the disease impact on health disparity, mainly in AA.

Disclosure

The Authors have no personal or financial conflicts of interest and have not entered into any agreement that could interfere with our access to the data on the research, or upon our ability to analyze the data independently, to prepare manuscripts, and to publish them.

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