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The Association of a Novel Identified *VDR* SNP With Prostate Cancer in African American Men

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Abstract. *Background/Aim: Vitamin D receptor (VDR) is present in numerous cellular pathways and it has been suggested that VDR genetic variants influence individual susceptibility to prostate cancer. Also, analyses of single nucleotide polymorphisms (SNPs) in VDR revealed ethnicity-associated polymorphisms. The aim of this study was to identify VDR SNPs in African American men with and without prostate cancer. Materials and Methods: The entire VDR gene was screened for germline mutations in a case-control study by denaturing high performance liquid chromatography and DNA sequencing. Logistic regression was used to estimate the association of SNPs, age, family history, and Gleason score with prostate cancer risk. Results: Six SNPs in the non-coding regions, and one SNP in the coding region, were detected. SNP 1 (c.278-69G>A) and SNP 4 (c.907+75C>T) have not been previously reported. SNP 4 had a significant protective effect (β=–0.6, $p<0.05$); whereas, SNP 7 (rs7975232) showed an increase association with prostate cancer risk and high Gleason score (β=0.32, $p<0.05$). SNP 4, SNP 7 and age were better predictors of prostate cancer risk than family history with a high degree of sensitivity (74.7%) and specificity (92.4%). Conclusion: SNP 4 and SNP 7 could be promising markers for prediction of reduced or increased prostate cancer risk, respectively.*

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Vitamin D (VD) has been reported as an important candidate implicated in the risk of prostate cancer (1-5). Although the exact mechanism of action is uncertain, various investigators have shown that serum 1,25-Dihydroxyvitamin D [1,25(OH)2 VD] levels can affect tumor cell proliferation and differentiation (6-12). Normal and malignant prostate cells contain VD receptors (VDRs) facilitate the anti-proliferative action of 1,25(OH)2 VD (13, 14).

The environmental and physiological factors which mediate the source of cutaneous VD are levels of ultraviolet radiation (UVR) exposure, skin-color, and genes involved in VD synthesis and metabolism (15-18). In our previous work, we described a marginally significant association between skin tanning and risk of prostate cancer. Whereas, no association between prostate cancer development and total UVR exposure or low VD status was noted (19). Analysis from our earlier study on cumulative UVR exposure per year and adult sunbathing scores derived from a validated questionnaire revealed a significant difference in cumulative sun exposure between prostate cancer cases and controls (20). Furthermore, outdoor and recreation UVR exposures were significantly higher in controls when compared to cases. The conditional logistic regression analysis indicated that there was no association between total UVR exposure and risk of prostate cancer after adjusting for age. Outdoor UVR exposure, however, was related with decreased prostate cancer risk. Additionally, we reported a propensity for reduced prostate cancer risk among men with early-life high sun exposure during childhood ages 0-5 years and 6-11 years. This inverse association between risk of prostate cancer and high early-life sun exposure intensity was also observed among young men at ages 12-17 years although not statistically significant. These results show that UVR exposure in early life may decrease prostate cancer risk. In addition to UVR exposure and skin-color, many publications reported that sequence variations within certain genes effect VD synthesis, action, and

metabolism. These genes include 1-alpha-hydroxylase, VD binding protein (*VDBP*) and *VDR* which are highly polymorphic among different human populations (21-24). Previously, we reported an association of *VDR* single nucleotide polymorphisms with prostate specific antigen (PSA) level, Gleason score, and prostate cancer risk in African-American men (21). There are various genetic studies that have investigated the relationship between *VDR* polymorphisms and prostate cancer risk (23, 25-33), many of which have suggested statistically significant associations (34-37), weaker associations (38, 39), and no associations (40-43) between common *VDR* variants and prostate cancer. However, those studies lack a consensus on how significantly, if at all, the VDR gene variants contribute to prostate cancer (44). Little is known about the association of *VDR* variants in high-risk populations, including African-American men with and without a family history of prostate cancer.

The present study was designed to evaluate the possibility that *VDR* genetic variants may contribute to prostate cancer risk. Therefore, the entire coding region and flanking introns of *VDR* were screened for germline mutations in a case-control study by denaturing high-performance liquid chromatography (DHPLC) followed by DNA sequencing.

Materials and Methods

Study population. Ninety-one African American men with histologically diagnosed adenocarcinoma of the prostate and 92 ethnicity matched (African American) controls were recruited through the ongoing free prostate cancer screening program at Howard University Cancer Center and from Howard University Hospital between 2005 and 2008. The Howard University Institutional Review Board (IRB-02-MED-42) approved the study protocol, and an informed consent was obtained from the study patients. Demographics and medical history details were previously described (19, 20).

Polymerase chain reaction (PCR). QIAmp DNA Blood Maxi Kit (Qiagen Inc., Valencia, CA, USA) was used to extract genomic DNA according to manufacturer's instructions. List of primers and PCR conditions were followed as previously described (45).

DHPLC. DHPLC instrument (WAVE® DNA Fragment Analysis System, Transgenomics, Omaha, NE, USA) equipped with a DNASep column (Transgenomic Inc., San Jose, CA, USA) was used to detect mutations and SNPs in *VDR* gene as previously described (45).

DNA sequencing and SNPs identification. Genomic DNA of samples demonstrating two or more heteroduplex peaks was amplified using GeneAmp 9700 thermal cycler as previously described (45) and purified by Qiagen column (QIAquick PCR purification Kit 50; Qiagen, Inc., Valencia, CA, USA). PCR was performed using 8 μl of Terminator ready reaction mix (Applied Biosystems, Foster City, CA, USA) in a 20 μl reaction volume containing 100 ng of PCR product as a template and 3.2 pmole PCR primers under the following conditions: denaturation at 94˚C for 4 min, 25 cycles of 30 s at 94˚C, 30 s at 50˚C, and 4 min at 60˚C, and a final extension step for 7 min at 60˚C. The PCR product was purified using Centriflex gel filtration cartridge (Edge Biosystems Inc., Gaithersburg, MD, USA); and the DNA pellet was washed with 70% ethanol, and suspended in 25 μl of Template Suppression Reagent (PE; P/N 401674), and denatured at 95˚C for 2 min. Sequencing was performed using ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA), and fluorescent labelled Big-dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) (46). Samples were also sequenced commercially by ACGT Incorporation (Wheeling, IL, USA) for confirmation. The generated data was analysed using Sequencher Version 4.8 software (Gene Codes Corporation), and SNPs were identified using International Hapmap project (http//: www.hapmap.org; https://www.ncbi.nlm.nih.gov/projects/SNP/). Nomenclature for the identified SNPs was assigned according to JT den Dunnen *et al*. (47), and the Reference SNP accession ID (rs ID) assigned for all previously reported SNPs using BLAST SNP (http://www.ncbi.nlm.nih.gov/SNP/snp_blastByOrg.cgi).

Statistical analyses. Cohen's d test was used to analyse the age distribution between prostate cancer cases and controls. Logistic regression analysis was used to assess the protective and high-risk SNPs on prostate cancer using prostate cancer as the outcome in relation to each individual SNP, and the effect of each individual SNP on prostate cancer while adjusting for the other SNPs. Logistic regression with Backward substitution was employed to determine the dominant SNPs that are associated with prostate cancer.

We further investigated the association of the best predictor SNPs, 4 and 7, with prostate cancer as an outcome and adjusting for the other risk factors (*e.g*., age, family history). Particularly, 4 models of risk factors were assessed: Model 1 [SNP4, SNP7 and family history], Model 2 [SNP4, SNP7 and age], Model 3 [SNP4, SNP7, family history and age], and Model 4 [age and family history (as a substitute for SNPs)].

The association of the SNPs, age, and family history with prostate cancer risk was also demonstrated using the area under the receiver-operating-characteristic curve (ROC; AUC) as a measure of prediction performance (Figure 1). The ROC was also used to determine the best model among the 4 models for predicting the risk of prostate cancer.

Each SNP was stratified by family history of prostate cancer and Gleason score as one of the composite variables that reflect prostate cancer aggressiveness. Gleason scores less than 7 and higher than 7 were considered as less and more advanced, respectively. Linear regression analysis was used to assess the association of *VDR* SNPs with Gleason score as the outcome using Model 5 (SNP 1, SNP 2, SNP 4, SNP 5, SNP 6, SNP 7, and Gleason score) and Model 6 (SNP 4, SNP 7, and Gleason score). All *p*-values less than 0.05 were considered statistically significant.

Results

Ninety-one African American men with prostate cancer and 92 control subjects were analyzed to identify SNPs in VDR gene. The mean age of prostate cancer and control subjects was 68.47 and 58.67 years; respectively. Cohen's d test of 1.05 indicated large effect size for average age difference between prostate cancer and control individuals (Table I).

Six distinct polymorphisms [SNP 1 (c.278-69G>A), SNP 2 (rs61614328), SNP 3 (rs11574114), SNP 4 (c.907+75

Table I. *Age distribution of prostate cancer patients (cases) and controls.*

	Number	Mean age±SD	Min-Max	Cohen's d [95% CI]
Total number	183	63.55±10.50	42-88	
Cases	91	68.47±9.09	47-87	1.05 [0.74,1.36]
Controls	92	58.67±9.51	42-88	

SD, Standard deviation; Min, minimum; Max, maximum; CI, confidence interval

Table II. *Identified vitamin D receptor (VDR) polymorphisms in African American men with (cases) or without prostate cancer (controls).*

	SNP[a]	dbSNP[b] identifier	Location in *VDR* gene	Controls n (%)[c]	Cases n (%)	Controls heterozygosity	Cases heterozigosity
1	c.278-69G>A	not reported	noncoding	32 (35)	37 (41)	0.17 (32/184)	0.20 (37/182)
2	c.755+25G>A	rs61614328	Intron 7/9	17 (19)	5 (5.5)	0.09 (17/184)	0.03 (05/182)
3	c.1025-95G>A	rs11574114	Intron 9	21 (23)	1 (1.1)	0.11 (21/184)	0.01 (01/182)
4	c.907+75C>T	not reported	Intron 8/9	66 (73)	28 (31)	0.36 (66/184)	0.15 (28/182)
5	c.1056T>C	rs731236	Exon 10	29 (32)	21 (23)	0.16 (29/184)	0.12 (21/182)
6	c.1025-56A>G	rs533037428	Intron 9	2 (2.2)	5 (5.5)	0.01 (02/184)	0.03 (05/182)
7	c.1025-49G>T	rs7975232	Intron 9	36 (40)	40 (44)	0.20 (36/184)	0.22 (40/182)

[a]Numbering nucleotide as previously described [47]. [b]SNP identifier based on NCB SNP database (http://www.ncbi.nlm.nih.gov/SNP/). [c]Altered allele frequency. SNP, Single-nucleotide polymorphism.

C>T), SNP 6 (rs533037428), and SNP 7 (rs7975232, *ApaI*)] in non-coding regions; and one distinct polymorphism in the coding region (SNP 5: rs731236, *TaqI*) have been detected in *VDR* (Table II). None of the SNPs were located in the conserved regions or splicing sites, or in the *VDR* promoter region. Only one nonsense polymorphism SNP 5 (a synonymous *Taq1* RFLP) in exon 9 was detected in the 3' coding region. Two of the detected polymorphisms, SNP 1 and SNP 4 have not been previously reported in the Entrez database SNP (dpSNP) and may be unique to African Americans. In our study, the prevalence of the variant alleles within SNP 1, SNP 2, SNP 3, SNP 4, SNP 5, SNP 6 and SNP 7 among cases was 35%, 19%, 23%, 73%, 32%, 2.2%, and 40%, respectively; and 41%, 5.5%, 1.1%, 31%, 23%, 5.5%, and 44% among controls, respectively (Table II).

Genotype and allele frequencies of SNP 2, SNP 3, SNP 5, SNP 6 and SNP 7 were previously reported in Hapmap database (http://hapmap.ncbi.nlm.nih.gov/index.html.en) and were different in African Americans than in Northern and Western Europeans (Table III). More specifically, frequencies of the altered alleles for SNP 2, SNP 3, SNP 5, SNP 6 and SNP 7 (0.50, 0.14, 0.23, 0.003 and 0.65; respectively) in African Americans were lower than in Europeans (0.0, 0.04, 0.44, 0.00 and 0.57; respectively).

A significant association of prostate cancer with SNP 2 (OR=0.26, 95% CI=0.09-0.73; p=0.011), SNP 3 (OR=0.04, 95% CI=0.005-0.29; p=0.002), and SNP 4 (OR=0.13, 95% CI=0.068-0.25; p<0.005); and a non-significant association of prostate cancer with SNP 1, SNP 5, SNP 6, and SNP 7 were found (p>0.005) (Table IV). SNP 1, SNP 6 and SNP 7 were associated with an increasing risk of prostate cancer (OR=1.12, OR=1.73, OR=1.28; respectively), although the association was not statistically significant (p>0.05). A statistically significant protective effect from prostate cancer risk was found for SNP 2 (OR=0.26, 95% CI=0.09-0.73; p=0.011), SNP 3 (OR=0.04, 95% CI=0.005-0.29; p=0.002), and SNP 4 (OR=0.13, 95% CI=0.068-0.25; p=0.000) (Table IV). SNP 3 was detected in only one prostate cancer case; therefore, it was excluded from further analysis.

When we used logistic regression analysis to study the effect of individual *VDR* SNPs on prostate cancer risk, we found that SNP 4 had a highly significant protective effect by reducing the risk of prostate cancer by 92% (OR=0.08, 95% CI=0.034-0.19; β=–2.52, p<0.05), while SNP 7 had a significant direct association by increasing the risk of prostate cancer by 6-fold (OR=6.03, 95% CI=2.077-17.509; β=1.80, p=0.001) when controlling for SNPs 1, 2, 5, and 6. Whereas, no significant associations were found between prostate cancer risk and SNP 1, SNP 5, and SNP 6 with (p>0.05) (Table V). When the 6 SNPs (SNP 1, SNP 2, SNP 3, SNP 4, SNP 5, SNP 6) were further analyzed using logistic regression with Backward substitution method, only SNPs 4 and 7 were the dominant SNPs associated with prostate cancer; and thus, the rest of the SNPs were dropped out from any further analysis. SNP 4 reduced the risk of prostate cancer by 92.4% (OR=0.076, 95% CI=0.033-0.172; β=-2.581, p=0.000) when

Table III. *Previously reported allele frequency and genotype in African Americans and Northern and Western Europeans.*

		Frequency of altered allele		Frequency of heterozygosity	
	SNP ID	AA	CEU	AA	CEU
SNP 2	rs61614328	0.50	0.00	1.00	0.00
SNP 3	rs11574114	0.14	0.04	0.72	0.07
SNP 5	rs731236	0.23	0.44	0.40	0.43
SNP 6	rs533037428	0.003	0.00	0.006	0.00
SNP 7	rs7975232	0.65	0.57	0.53	0.40

SNP, Single-nucleotide polymorphism; CEU, Utah residents with ancestry from Northern and Western Europeans; AA, African Americans.

Table IV. *Association of individual vitamin D receptor (VDR) single-nucleotide polymorphisms (SNPs) with prostate cancer risk (1=altered, 0=wild type).*

Variants		Cases (total number)	Controls (total number)	Odd ratio [95% CI]	*p*-Value
SNP 1:	1	35	33	1.12 [0.61-2.04]	0.72
	0	56	59		
SNP 2:	1	5	17	0.26 [0.09-0.73]	0.011
	0	86	75		
SNP 3:	1	1	21	0.04 [0.005-0.29]	0.002
	0	90	71		
SNP 4:	1	28	71	0.13 [0.068-0.25]	1.66E-09
	0	63	21		
SNP 5:	1	21	29	0.65 [0.34-1.26]	0.201
	0	70	63		
SNP 6:	1	5	3	1.73 [0.4-7.44]	0.465
	0	86	89		
SNP 7:	1	40	35	1.28 [0.71-2.31]	0.42
	0	51	57		

adjusting for SNP 7. Moreover, SNP 7 increased the risk by 3.6-fold (OR=3.632, 95% CI=1.598-8.254; β=1.290, p=0.002) when adjusting for SNP 4 (Table VI).

Furthermore, logistic regression analysis was used to examine whether the effect of the best predicted SNPs 4 and 7 is modulated by another factor (such as age, family history, Gleason score) (Table VII). In particular, we assessed "SNP 4, SNP 7, family history"; "SNP 4, SNP 7, age"; "SNP 4, SNP 7, family history, age"; "Age, family history"; in 4 models (Model, 1, Model 2, Model 3, and Model 4; respectively). Models 1, 2, and 3 showed that SNP 4 and SNP 7 maintained their protective effect and risk association with prostate cancer; respectively. Model 4 showed a marginal significant association of family history with prostate cancer risk when adjusting for age. As expected, an increased significant association of age and family history with prostate cancer risk was found (p<0.05).

The statistically significant association of the SNPs, age, and family history with prostate cancer risk was also demonstrated using the AUC as a measure of prediction performance. The overall accuracy expressed by the AUC value increased from 77.9% for age and family history (Model 4) to 86% when the SNPs 4 and 7 were included in the prediction model (Model 3) for prostate cancer risk. Similarly, an increase from 75.1% for SNP 4, SNP 7 and family history (Model 1) to 86% was observed when the age was included (Model 3).

The ROC curves and AUC values (Figures 1 and 2) showed that SNP 4, SNP 7, and age were better predictors (AUC=85.8%) than family history in predicting prostate cancer risk with a high degree of sensitivity (74.7%), and specificity (92.4%) for the optimal cut-off point for Model 2. The combination of SNP 4, SNP 7, family history and age had a higher AUC value (86%) than the age and family history (AUC=77.9%), or SNP 4, SNP 7 and family history (AUC=75.1%).

The association between *VDR* SNPs and Gleason score was also determined using linear regression in two Models

Table V. *The association of individual single-nucleotide polymorphisms (SNPs) with prostate cancer while adjusting for other SNPs using logistic regression.*

Independent	β	Standard error	*p*-Value	Odd ratio	95% CI
SNP 1	0.427	0.38	0.258	1.53	0.731-3.212
SNP 2	–0.72	0.62	0.244	0.49	0.145-1.635
SNP 4	–2.52	0.44	7.7E-09	0.08	0.034-0.19
SNP 5	–0.83	0.52	0.111	0.44	0.158-1.208
SNP 6	0.993	0.90	0.272	2.70	0.458-15.889
SNP 7	1.797	0.54	0.001	6.03	2.077-17.509
Constant	0.726	0.29	0.011	2.07	

Table VI. *Assessment of association of the most predictive vitamin D receptor (VDR) single-nucleotide polymorphisms (SNPs) with prostate cancer risk using logistic regression analysis with Backward substitution method (p≤0.05).*

Independent	β	Standard error	*p*-Value	Odd ratio	95% CI
SNP4	–2.581	0.418	0.000	0.076	0.033-0.172
SNP7	1.290	0.419	0.002	3.632	1.598-8.254
Constant	0.855	0.263	0.001	2.351	

5 and 6 (Table VIII). Gleason score as the response variable and SNPs 1, 2, 3, 4, 5, 6 and 7 as the predictors of prostate cancer. Model 5, the effect of SNP 7 after adjusting for all other SNPs (SNP 1, SNP 2, SNP 4, SNP 5, SNP 6, SNP 7, and Gleason score); and Model 6, the effect of each SNP at the level of the other SNPs (SNP 4, SNP 7, and Gleason score). The significant results of Models 5 and 6 indicate that SNP 4 (β=–0.64, $p<0.05$) had a significant protective effect against prostate cancer and was associated with a low Gleason score. On the other hand, SNP 7 showed a statistically significant association with increased prostate cancer risk and high Gleason score (β=0.32, $p<0.05$).

Discussion

Common genetic variants in *VDR* have been related to prostate cancer risk in several studies, but the overall results remain contradictory. Two initial studies showed a 3 to 4-fold increase risk of prostate cancer associated with *VDR* polymorphisms in the 3' end of the gene (23, 25). However, a meta-analysis involving 17 studies that assessed the *TaqI* (rs731236), *BsmI*, and *poly-A* repeat polymorphisms, as well as the *FokI* polymorphism in exon 2, concluded that none of these variants was likely to be a major determinant of prostate cancer risk (48). Further, it has been suggested that *VDR* SNPs may be more related to advanced disease (23, 26, 49-51). Earlier investigations including cases with localized

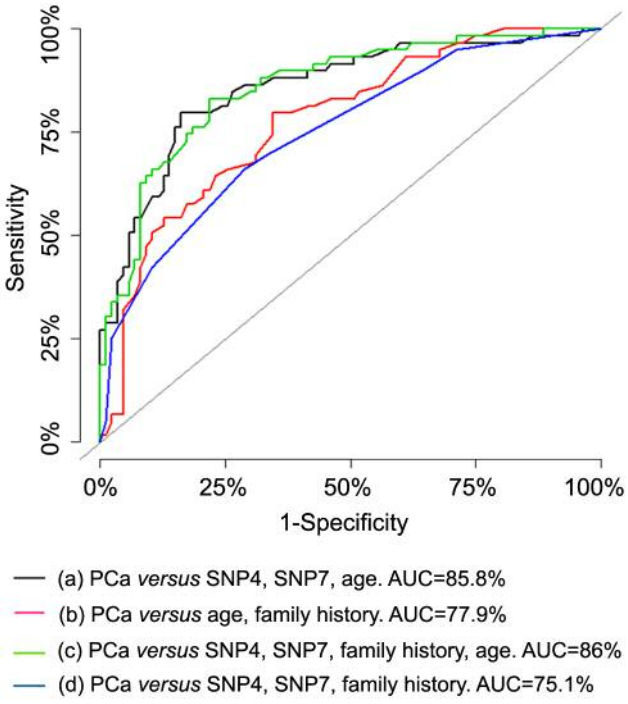

Figure 1. *Area under the curve (AUC) for the receiver-operating-characteristic curve analysis for sensitivity and specificity of the models: (a) Prostate cancer (PCa) versus SNP4, SNP7, age; (b) PCa versus age, family history; (c) PCa versus SNP4, SNP7, family history, age; (d) PCa versus SNP4, SNP7, family history.*

Table VII. *Association of vitamin D receptor (VDR) single-nucleotide polymorphisms (SNPs) and Gleason score. (p≤0.05).*

Logistic Regression: Prostate cancer as the outcome					95% CI	
Independent	Beta	Standard error	*p*-Value	Odd ratio	Lower	Upper
Model 1						
SNP 4	–2.525	0.420	1.82E-9	0.080	0.035	0.182
SNP 7	1.349	0.422	1.38E-3	3.855	1.687	8.809
Family	0.309	0.404	4.44E-1	1.362	0.617	3.005
Constant	0.736	0.288	1.06E-2	2.088		
Model 2						
SNP 4	–2.780	0.486	1.05E-8	0.062	0.024	0.161
SNP 7	1.613	0.483	8.4E-4	5.020	1.947	12.942
Age	0.121	0.023	1.11E-7	1.129	1.080	1.181
Constant	–6.950	1.474	2.42E-6	0.001		
Model 3						
SNP 4	–2.687	0.488	3.58E-8	0.068	0.026	0.177
SNP 7	1.637	0.486	7.56E-4	5.142	1.983	13.330
Family	0.490	0.453	2.8E-1	1.632	0.671	3.964
Age	0.121	0.023	1.64E-7	1.128	1.078	1.180
Constant	–7.082	1.508	2.67E-6	0.001		
Model 4						
Age	0.112	0.020	1.351E-8	1.118	1.076	1.162
Family	0.774	0.399	0.052	2.168	0.992	4.738
Constant	–7.301	1.283	1.250E-8	0.001		

Table VIII. *Association of the two most predictive vitamin D receptor (VDR) single-nucleotide polymorphisms (SNPs), family history, and age with Gleason score using linear regression analysis.*

Linear regression: Gleason score as the outcome				95% CI	
Independent	Beta	Standard error	*p*-Value	Lower	Upper
Model 5					
SNP 1	0.030	0.133	0.822	–0.232	0.292
SNP 2	–0.137	0.193	0.479	–0.517	0.244
SNP 4	–0.600	0.141	3.5e-5	–0.878	–0.322
SNP 5	–0.239	0.188	0.205	–0.610	0.132
SNP 6	0.173	0.363	0.635	–0.545	0.890
SNP 7	0.455	0.174	0.010	0.111	0.799
Constant	0.823	0.114	0.000	0.597	1.049
Model 6					
SNP 4	–0.64	0.134	4.0E-6	–0.908	–0.378
SNP 7	0.32	0.133	0.017	0.057	0.581
Constant	0.837	0.106	0.000	0.628	1.046

and advanced disease revealed a reduced risk of prostate cancer related with the *TaqI t* allele or an allele in linkage disequilibrium (LD) with *TaqI* t (52-58). Among Japanese populations, *ApaI* (rs7975232) was not significantly associated with either familial prostate cancer (59) or sporadic prostate cancer and benign prostatic hyperplasia (60). Prostate cancer has been inversely associated with the TaqI tt genotype in White and Black men in North Carolina (25). Similarly, it was reported by Ma *et al*. (49) that reduced prostate cancer risk is associated with the *TaqI* tt genotype; however, only among men with low serum 1,25 (OH)2 VD levels. In a recent meta-analysis, it was reported that VDR *TaqI* polymorphism may be associated with prostate cancer risk in the Asian population (37), and particularly in the Japanese population (24). Although, *VDR* SNPs have been evaluated as markers of prostate cancer risk; their impact remains uncertain especially in African-Americans. In the current study, we identified and evaluated seven SNPs in the

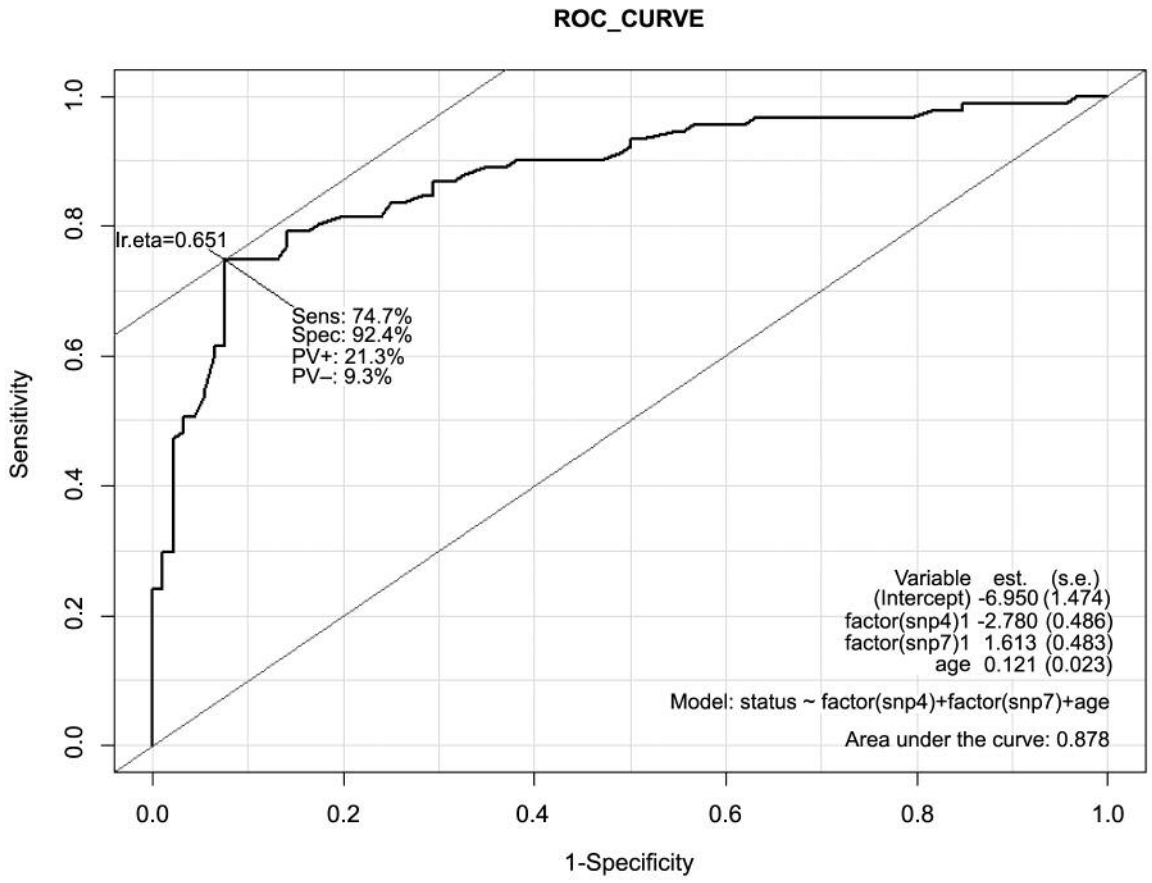

Figure 2. *Validation of the best predictive model for prostate cancer using the area under the receiver-operating-characteristic curve as a measure of prediction performance.*

VDR gene which had a significant association or no association with the risk of prostate cancer. Two of the SNPs, 1 and 4, were not reported in the SNPs database. Thus, we postulate that these two SNPs are novel. Moreover, the present study is the first to show SNP 4 as a determinant of prostate cancer risk.

Two of the detected SNPs, SNP 5 (*Taql*) and SNP 7 (*Apal*), have been previously reported (48, 61). In our previous study (21), SNP 5 was found to be significantly associated with prostate cancer risk in African American men after adjusting for age using the codominant, dominant, and the log-additive models; whereas, SNP 7 was significantly associated with an increased prostate cancer risk using the recessive model. There was no evidence that these associations were modified by family history of prostate cancer, which is inconsistent with the present study. Moreover, we have previously investigated whether DHPLC elution patterns of *VDR* PCR products can serve as indicators of susceptibility to prostate cancer risk (45). A similar elution pattern of exons 1, 6, 7 and 9 along with the higher prevalence of heteroduplex DNA in prostate cancer cases than in controls was observed. Moreover, exons 4 and 8 had highly significant protective effects against prostate cancer. On the other hand, exons 5, 7, and 9 were positively correlated with prostate cancer risk, thus they could be better predictors of susceptibility to prostate cancer. This data is also inconsistent with our present study in which SNP 4 (Intron 8/9) showed a protective effect for prostate cancer development, while SNP 7 (Intron 9) was associated with increased risk of prostate cancer.

Both Gleason score and TNM stage are accepted as valid and reliable measures for assessing the aggressiveness of prostate cancer (62). However, Gleason score was found to be a more sensitive measure of aggressive prostate cancer than TNM stage (59, 61, 63), and the associations of *VDR* variants with Gleason grade were identified (64). In the present study, we found that carriers of the variant SNP 4 had a significant protective effect and low Gleason score of prostate cancer (β=–0.6, p<0.05); whereas, carriers of SNP

7 had higher risk and poorly differentiated prostate cancer (β=0.32, p<0.05). Further large studies that replicate these results are warranted to confirm the association of SNPs 4 and 7 with prostate cancer.

It is speculated that a substitute for long-term serum VD levels is the level of UVR exposure. Prostate cancer and control patients classified into low- and high- exposure group based on cumulative UVR exposure may mask any effect of *VDR* variants in men with different VD levels (23). Several publications reported that the pathogenesis of prostate cancer in men with low levels of UVR exposure is different from that in men with higher levels (8, 65-67). However, if the functional differences between the *VDR* genotypes are small relative to the consequences of low vitamin levels, the effect of the polymorphisms may be concealed. In contrast, men with UVR exposure above the median would be expected to synthesize adequate amounts of VD. The functional consequences of the polymorphisms may be sufficiently great in the presence of an adequate concentration of the VD to influence prostate cancer risk. Thus, the VD pathway may have an etiologic role in the development of prostate cancer.

Conclusion

In African American men, SNP 4, SNP 7, and age were better predictors of prostate cancer risk than family history. Moreover, our results suggested that SNP 4 and 7 could be promising predicting markers of reduced or increased prostate cancer risk, respectively. Further, larger studies are warranted to confirm the association of these markers with prostate cancer.

Authors' Contributions

MD and DB conducted the experiments. VA performed the statistical analyses. TN collected the clinical data and contributed to data analyses. OOK contributed to the data analyses and manuscript writing. RLC and YK were responsible for the experimental design and contributed to the data analyses and manuscript writing

Conflicts of Interest

The Authors have no personal or financial conflicts of interest.

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