SPINK1 Promoter Variants Are Associated with Prostate Cancer Predisposing Alterations in Benign Prostatic Hyperplasia Patients

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Abstract. *Background/Aim: Several studies reported that patients with benign prostatic hyperplasia (BPH) experienced a 10% increased incidence of prostate cancer (PCa) after the first 5 years of diagnosis. We investigated the association between single nucleotide polymorphisms (SNPs) in the promoter of Serine Protease Inhibitor Kazal Type 1 (SPINK1) and the increased risk of BPH and PCa. Materials and Methods: We genotyped three SNPs in a cases-control study, including BPH and PCa cases. Multiple logistic regression models were applied to analyze clinical and genotypic data. Results: We found an inverse association between SNP rs10035432 and BPH under the log-additive (p=0.007) model. No association was found between these SNPs and PCa risk. However, we observed a possible association between rs1432982 and lower-grade PCa (p=0.05) under the recessive model. Conclusion: SPINK1 promoter variants are likely to be associated with the risk of BPH.*

Benign prostatic hyperplasia (BPH) is a commonly diagnosed disease in men above 50 years of age (1-3). It refers to the non-malignant growth of the prostate and is defined as prostate gland enlargement secondary to hyper-proliferation of stromal and granular cells with predominance of mesenchymal cells (4). The etiology and pathogenesis of BPH is still poorly understood (5). Many factors have been reported to play a role in the development of BPH, including inflammatory genes, inflammatory mediators, hormones, dietary factors and oxidative stress (6). Additional risk factors include a positive family history of BPH, genetic polymorphisms and early age of onset of BPH (7-11). Another prostatic disease, prostate cancer (PCa), is the second leading cause of cancer-related deaths among men in the United States (12). Chokkalingam *et al*. reported that patients with BPH experienced a 10% excess incidence of PCa after the first 5-years of follow-up (13). Several researchers reported a genetic association between BPH development and polymorphisms of specific genes expressed in the prostate (14-16). In a recent study, Väänänen *et al*. reported that Serine Protease Inhibitor Kazal Type 1 (SPINK1) expression was detected in histologically benign-area from PCa specimens (17). However, there exists limited information about single nucleotide polymorphisms (SNPs) in the *SPINK1* gene association with the risk of BPH.

In addition, previous studies have shown that SPINK1 is over-expressed in PCa tissues, especially high-grade tumors (Gleason score >7) (18, 19). SPINK1 over-expression exclusively occurs in a subset of PCa tissues without E26 transformation-specific (*ETS*) gene fusion suggesting that *SPINK1*-positive tumors may represent a separate PCa sub-type (20-22). In a urine-based analysis, *SPINK1* showed a significant association for discriminating between men with or without PCa disease and had a greater specificity for diagnosing PCa than prostate-specific antigen (PSA) (23). Recently, it was reported that SPINK1 over-expression is more common in African American (AA) PCa tissues compared to

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Caucasian men (24). Although previous studies have implicated *SPINK1* as a molecular biomarker for a subset of PCa cases, the exact functional role of SPINK1 in PCa is unclear. However, it has been speculated that *SPINK1* over-expression is a consequence of upstream genetic events (20).

SPINK1 is located on chromosome 5q32, encodes a 56-amino acid peptide, which functions as an acute-phase protein (APP) in the pancreas (25). Initially, SPINK1 was thought to prevent premature activation of trypsinogen (inactive form of trypsin), thereby ensuring the integrity of acinar cells to prevent auto-digestion of the pancreas (26-28). Some studies have reported *SPINK1* to play a role in tissue repair, apoptosis, reproduction and behave as a growth factor in other human organs (29-33).

The promoter of *SPINK1* contains a consensus sequence "TTGNNGNAATG" for the nuclear factor-interleukin six (NF-IL6) binding site, known as the IL-6 response element (IL-6-RE) (25). This sequence is a 40-bp DNA fragment located between -3.84 kb and -3.80 kb upstream from the start site (25). This fragment is conserved among various acute-phase genes and is responsible for both *SPINK1* transcriptional activity and IL-6-induced expression of *SPINK1* (34, 35). Given that IL-6 expression is associated with aggressive PCa phenotypes, this transcription factor site may be involved in the gain-of-function of SPINK1 in PCa tissue (36-38). Still, the pathological relevance of the known *SPINK1* promoter variants is largely unknown. Therefore, we hypothesize that polymorphic variations in the IL-6RE of the *SPINK1* gene may be associated with increased risk of BPH and PCa. To approach our hypothesis, we (i) genotyped three known single nucleotide polymorphisms (SNPs) in *SPINK1* promoter in PCa patients, sub-group of men diagnosed with BPH and controls, (ii) performed a functional *in silico* analysis to determine the functionality of the tested *SPINK1* promoter variants and (iii) integrated the resulting data with the clinical and pathological characteristics in order to assess the pathological relevance of each SNP.

Materials and Methods

Study population. This is a case-control study comprised of 2 selected cohorts from the "African American (AA) Sporadic PCa" study (AAPCA) and the "Vitamin D and PCa Risk in AA Men" studies. These studies were conducted at the National Human Genome Center (NHGC) and Howard University Cancer Center (HUCC). The Howard University Institutional Review Board (IRB) approved the study protocols (IRB-02-MED-42; IRB-11-MED-22) and written informed consent was obtained from all study subjects. Briefly, participants were recruited from the Division of Urology at the Howard University Hospital (HUH) and/or from ongoing free PCa screening program at the HUCC. From the above mentioned two studies, we selected (i) 307 non-related men of African descent (*i.e.* self-reported AA, Afro-Caribbean, African Latinos, East and West Africans) 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); (ii) 96 subjects diagnosed with BPH based on the clinical symptoms, such as scrotal discomfort and urination problems; and (iii) control subjects (n=339) with PSA levels <4.0 ng/ml, normal DRE and with no history of PCa among first-degree relatives. Blood samples were drawn from each participant and clinical characteristics, such as Gleason score (high and low Gleason score were defined as less advanced "if a case's Gleason score is <7" and more advanced "if their Gleason score is >7") and PSA level were obtained from the HUH Tumor Registry and pathology records.

SNP selection. The SNPs in the IL-6 response element in the promoter region of *SPINK1* were selected using National Center for Biotechnology Information (NCBI) (39) and International Haplotype Map (HapMap) (40). Utilizing data from Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Yoruba in Ibadan, Nigeria (YRI) population, a Chi-square test was performed to determine significant differences in minor allele frequencies (MAF) within the populations. Haploview version 4 was utilized for selecting the tagSNPs in IL-6 response element in the *SPINK1* promoter region (41). The selection parameters were set to capture MAF of 5% or more and r^2 values of 0.8 or more in the promoter region of *SPINK1*. A total of 56 *SPINK1* promoter SNPs have been reported in HapMap. Out of these 56 SNPs, 15 were located in the NF-IL-6 binding site. Out of these 15 SNPs, four had minor allele frequencies of 10% or greater, which was needed for adequate power. Of the four SNPs, three variants, rs10035432, rs11748222 and rs1432982, showed significant differences in MAF between CEU and YRI and had adequate power for the sample set.

Genotyping. Genomic DNA was extracted from lymphocytes using the Blood and Cell Culture DNA Midi Kit (Qiagen, Germantown, MD, USA) in accordance with the manufacturer's instructions. Twenty ng of DNA was used in each predesigned TaqMan® 5' allelic discrimination assay (Life Technologies, Grand Island, NY, USA) for genotyping the 3 selected SNPs. For quality control, subjects were genotyped in duplicate and the overall concordance rate was 99.9%. Genotyping assays of the *SPINK1* promoter SNPs (Table I) were performed using Applied Biosystems 7900HT Real-Time PCR System with TaqMan SNP assay reagents. The ABI 7900HT Sequence Detection System (SDS) software (version 2.0) was used to analyze real-time, end-point fluorescence and allelic discrimination (Life Technologies, Grand Island, NY, USA).

In silico analysis to study the functional effects of the SNPs. The Alibaba 2.1 software (42) was utilized to determine the functionality of the tested *SPINK1* promoter variants and their pathological relevance; and if any of the tested SNPs disrupted or resulted in the appearance of putative transcription factors binding site.

Statistical analyses. The statistical analyses were performed using R (43) and SNPStats (44). Allele frequencies in controls were tested for Hardy-Weinberg equilibrium using chi square analysis and Fisher's exact test, when appropriate. The association of disease status and clinical phenotypes with genotype and haplotype was analyzed by binary and multivariate logistic regression. For each SNP genotype, tests using five different genetic models: co-dominant, dominant, recessive, over-dominant and log-additive, were performed. Multiple logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI).

Table I. *TaqMan® SNP genotyping assays identification (ID) and probe sequences.*

SNP ID	Genotyping Assays ID	*Context Sequence [VIC/FAM]
rs10035432	C__30608242_20	TAATTGAGCTTTAGCTATAAAATCC[A/G]TATATATTCCTCTGAAGCTTAAATA
rs11748222	C___3057756_20	TCTGATACATCAAAAACACCCAGCA[C/T]GTTGGTTATTATTCTTTTTATTGAT
rs1432982	C___8950584_10	AAACAAATATAAAGACAATTTAAAA[A/G]TGCATGAGCATAGTAAAGCTTGACG

*Each designed TaqMan® SNP Genotyping Assay includes two allele-specific TaqMan® MGB probes containing distinct 5' fluorescently-labeled single primers and minor groove binder (MGB) non-fluorescent quencher (NFQ) on the 3' end, as well as 2 unlabeled PCR primers (forward and reverse) to detect specific SNP targets. One VIC® dye - MGB-labeled probe detects the Allele 1 sequence and one 6FAM™ dye - MGB-labeled probe detects the Allele 2 sequence. FAM (6-carboxyfluorescein)/ VIC (4, 7, 2'-trichloro-7'-phenyl-6-carboxyfluorescein).

Table II. *Characteristics of study subjects.*

	Controls	Cases	*BPH	**p-Value
Total number of participants	339	307	96	
Mean age (±SD)	56.59±10.33	67.84±10.02	63±9.64	0.0001
Mean PSA level	1.20±0.87	74.51±383.52	8.74±20.6	0.0005
Mean Gleason Score				
<7	-	5.44±0.07	-	
>7	-	8.80±0.12	-	
History of WAA (%)	67.33%	70.77%	68.2%	0.02

Plus-minus values are means±SD. Prostate-specific antigen (PSA) levels (ng/ml) were obtained at the time of diagnosis for cases and at the time of study enrollment for the controls. *Benign prostatic hyperplasia (BPH). **p-value (*t*-Test, 2-tailed). WAA, West African ancestry.

Two-sided *p*-values of ≤0.05 were considered as statistically significant. ORs for associations between SNPs, Gleason score and PCa risk were adjusted for age and percentage of West African ancestry. ORs for associations between SNPs and BPH were adjusted for age. To test the combinatorial effects of the SNPs and haplotypes, SNPStats was utilized (44).

Results

Participants' demographics. In this study, 307 PCa and 96 BPH patients, as well as 339 control subjects, were investigated in order to explore the association of polymorphic variations in the IL-6RE of *SPINK1* gene with BPH and PCa risk. The characteristics of the human subjects are shown in Table II. A student's *t*-test reveals significant differences in age between the PCa, BPH and control subjects (*p*<0.0001). The mean age of patients with PCa (67.84±10.02) was significantly older than that of controls (56.59±10.33) and BPH patients (63±9.6). The mean PSA levels were significantly higher in PCa (74.51 ng/ml) and BPH (8.74 ng/ml) patients than controls (1.20 ng/ml) (*p*<0.0005). The mean Gleason Score with ranges 2-6 and 7-10 in PCa cases was 4.44±0.07 and 8.80±0.12, respectively.

The percentage of subjects with West African ancestry was significantly different between PCa (70.77%) subjects and controls (67.33%) (*p*=0.02) and not in BPH subjects (68.2%).

Associations between SNPs, Gleason score and BPH risk. The studied rs10035432, rs11748222 and rs1432982 SNPs were in the Hardy-Weinberg equilibrium (*p*>0.05). The allele and genotype frequencies in PCa patients were similar to the controls. The majority of PCa patients were homozygous for the major alleles of rs10035432, rs11748222 and rs1432982 SNPs (Table III). For SNP rs10035432 the G/G genotype was 58% and 58.5% among cases and controls, respectively. The T/T genotype of rs11748222 was 80.8% among cases and 79% among controls. For SNP rs1432982, the A/A genotype was 61.5% among cases compared to 58.5% among controls.

None of the studied SNPs were found to be associated with PCa risk even after adjusting for age and %WAA using the five genetic models (*p*>0.05) (Table III). The association between the three SNPs and Gleason score was also determined (Table IV). We found a significant association between the minor allele G of rs1432982 with low Gleason score and PCa risk after adjusting for age under the recessive model (OR=2.96, 95% CI=0.91-9.61; *p*=0.05). *In silico* analysis of rs1432982 revealed that when the G allele is present, there is a CCAAT-enhancer-binding protein alpha (c-EBPα) site; however when the A allele is present the c-EBPα site is absent. We found an association between the A allele of rs10035432 and BPH

Table III. *Association between SPINK1 SNPs and prostate cancer risk.*

			Rs10035432		
Model	Genotype	Controls	*Cases	**OR (95% CI)	***p-Value
Co-dominant	G/G	65 (58%)	55 (58.5%)	1	0.44
	A/G	44 (39.3%)	33 (35.1%)	0.87 (0.44-1.71)	
	A/A	3 (2.7%)	6 (6.4%)	0.35 (0.07-1.81)	
Dominant	G/G	65 (58%)	55 (58.5%)	1	0.50
	A/G-A/A	47 (42%)	39 (41.5%)	0.80 (0.41-1.54)	
Recessive	G/G-A/G	109 (97.3%)	88 (93.6%)	1	0.22
	A/A	3 (2.7%)	6 (6.4%)	0.38 (0.08-1.87)	
Over-dominant	G/G-A/A	68 (60.7%)	61 (64.9%)	1	0.87
	A/G	44 (39.3%)	33 (35.1%)	0.95 (0.49-1.83)	
Log-additive	---	---	---	0.75 (0.43-1.31)	0.31

			rs11748222		
Model	Genotype	Controls	*Cases	**OR (95% CI)	***p-Value
Co-dominant	T/T	83 (79%)	63 (80.8%)	1	0.97
	C/T	15 (14.3%)	10 (12.8%)	1.10 (0.40-3.01)	
	C/C	7 (6.7%)	5 (6.4%)	0.90 (0.23-3.49)	
Dominant	T/T	83 (79%)	63 (80.8%)	1	0.96
	C/T-C/C	22 (20.9%)	15 (19.2%)	1.02 (0.44-2.40)	
Recessive	T/T-C/T	98 (93.3%)	73 (93.6%)	1	0.86
	C/C	7 (6.7%)	5 (6.4%)	0.89 (0.23-3.42)	
Over-dominant	T/T-C/C	90 (85.7%)	68 (87.2%)	1	0.85
	C/T	15 (14.3%)	10 (12.8%)	1.10 (0.40-3.02)	
Log-additive	---	---	---	0.99 (0.55-1.79)	0.97

			rs1432982		
Model	Genotype	Controls	*Cases	**OR (95% CI)	***p-Value
Co-dominant	A/A	69 (58.5%)	59 (61.5%)	1	0.48
	A/G	42 (35.6%)	27 (28.1%)	1.16 (0.59-2.30)	
	G/G	7 (5.9%)	10 (10.4%)	0.53 (0.15-1.82)	
Dominant	A/A	69 (58.5%)	59 (61.5%)	1	0.99
	A/G-G/G	49 (41.5%)	37 (38.5%)	1.00 (0.53-1.90)	
Recessive	A/A-A/G	111 (94.1%)	86 (89.6%)	1	0.26
	G/G	7 (5.9%)	10 (10.4%)	0.50 (0.15-1.69)	
Over-dominant	A/A-G/G	76 (64.4%)	69 (71.9%)	1	0.52
	A/G	42 (35.6%)	27 (28.1%)	1.25 (0.64-2.43)	
Log-additive	---	---	---	0.89 (0.54-1.46)	0.65

*Cases, included only PCa cases. **OR (95% CI): odd ratio and 95% confidential interval of SNP response to PCa when adjusted by age and %West African ancestry. ***p-Value, adjusted p-value by age.

risk before adjusting for age (data not shown) and even after adjusting for age and %WAA under the co-dominant (OR=0.52, 95% CI=0.29-0.93; *p*=0.02), dominant (OR=0.48, 95% CI=0.28-0.84; *p*=0.01) and the log-additive (OR=0.55, 95% CI=0.35-0.84; *p*=0.007) models (Table V). *In silico* analysis of rs10035432 showed that when the A allele is present there is a TATA Binding Protein (TBP) site; however, when the G allele is present this site is lost.

A haplotype analysis of the three studied SNPs revealed only one associated haplotype with low Gleason score and PCa risk (OR=2.01, 95% CI=1.00-4.04; *p*=0.05). This haplotype is composed of the rs10035432 G allele, rs11748222 T allele and rs1432982 G allele (Table VI). Whereas, an inverse association was found between haplotype 2 (rs10035432 A allele, rs11748222 T allele and rs1432982 A allele) and BPH risk (OR=0.63, 95% CI=0.38-1.06; *p*=0.08) (Table VII).

Table IV. *SPINK1 SNPs association with Gleason score (GS) in prostate cancer cases*.

		rs10035432			
Model	Genotype	*Low GS	*High GS	**OR (95% CI)	***p-Value
Co-dominant	G/G	50 (65.8%)	42 (60.9%)	1	0.48
	A/G	18 (23.7%)	22 (31.9%)	0.69 (0.33-1.45)	
	A/A	8 (10.5%)	5 (7.2%)	1.34 (0.41-4.42)	
Dominant	G/G	50 (65.8%)	42 (60.9%)	1	0.54
	A/G-A/A	26 (34.2%)	27 (39.1%)	0.81 (0.41-1.59)	
Recessive	G/G-A/G	68 (89.5%)	64 (92.8%)	1	0.49
	A/A	8 (10.5%)	5 (7.2%)	1.51 (0.47-4.84)	
Over-dominant	G/G-A/A	58 (76.3%)	47 (68.1%)	1	0.27
	A/G	18 (23.7%)	22 (31.9%)	0.66 (0.32-1.38)	
Log-additive	---	---	---	0.96 (0.58-1.58)	0.88

		rs11748222			
Model	Genotype	*Low GS	*High GS	**OR (95% CI)	***p-Value
Co-dominant	T/T	67 (80.7%)	66 (84.6%)	1	0.54
	C/T	11 (13.2%)	10 (12.8%)	1.08 (0.43-2.72)	
	C/C	5 (6%)	2 (2.6%)	2.46 (0.46-13.14)	
Dominant	T/T	67 (80.7%)	66 (84.6%)	1	0.51
	C/T-C/C	16 (19.3%)	12 (15.4%)	1.31 (0.58-2.99)	
Recessive	T/T-C/T	78 (94%)	76 (97.4%)	1	0.27
	C/C	5 (6%)	2 (2.6%)	2.44 (0.46-12.94)	
Over-dominant	T/T-C/C	72 (86.8%)	68 (87.2%)	1	0.94
	C/T	11 (13.2%)	10 (12.8%)	1.04 (0.41-2.60)	
Log-additive	---	---	---	1.34 (0.72-2.50)	0.36

		rs1432982			
Model	Genotype	*Low GS	*High GS	**OR (95% CI)	***p-Value
Co-dominant	A/A	50 (60.2%)	50 (67.6%)	1	0.16
	A/G	21 (25.3%)	20 (27%)	1.05 (0.51-2.17)	
	G/G	12 (14.5%)	4 (5.4%)	3.00 (0.91-9.94)	
Dominant	A/A	50 (60.2%)	50 (67.6%)	1	0.34
	A/G-G/G	33 (39.8%)	24 (32.4%)	1.37 (0.71-2.65)	
Recessive	A/A-A/G	71 (85.5%)	70 (94.6%)	1	0.05
	G/G	12 (14.5%)	4 (5.4%)	2.96 (0.91-9.61)	
Over-dominant	A/A-G/G	62 (74.7%)	54 (73%)	1	0.81
	A/G	21 (25.3%)	20 (27%)	0.91 (0.45-1.87)	
Log-additive	---	---	---	1.45 (0.90-2.34)	0.13

*Low and high GS were defined as less advanced "if a case's GS is <7" and more advanced "if their GS is >7". **OR (95% CI): odd ratio and 95% confidential interval of SNP response to PCa when adjusted by age. ***p-Value, adjusted p-value by age.

Discussion

In the present study, we investigated whether SNPs in *SPINK1* are associated with an increased risk of BPH and PCa. We identified and genotyped a sub-set of population frequency discriminant alleles in the IL-6 response element (IL-6 RE) of the *SPINK1* gene (rs10035432, rs11748222 and rs1432982) in PCa cases, controls and patients diagnosed with BPH, who were of African descent. None of the investigated *SPINK1* SNPs was associated with PCa risk. However, we found a significant association between the minor allele G of rs1432982, low GS under the recessive model. We did observe an inverse association between rs10035432 and BPH under the log additive model.

Though *SPINK1* is being targeted as a biomarker for PCa, there exists limited information regarding the association between the genetic variants present in this gene and PCa risk. To our knowledge we are the first to

Table V. *Associations between SPINK1 SNPs and benign prostatic hyperplasia (BPH) risk.*

			Rs10035432		
Model	Genotype	Controls	BPH	*OR (95% CI)	**p-Value
Co-dominant	G/G	165 (58.1%)	30 (44.1%)	1	0.02
	A/G	106 (37.3%)	31 (45.6%)	0.52 (0.29-0.93)	
	A/A	13 (4.6%)	7 (10.3%)	0.32 (0.11-0.90)	
Dominant	G/G	165 (58.1%)	30 (44.1%)	1	0.01
	A/G-A/A	119 (41.9%)	38 (55.9%)	0.48 (0.28-0.84)	
Recessive	G/G-A/G	271 (95.4%)	61 (89.7%)	1	0.11
	A/A	13 (4.6%)	7 (10.3%)	0.43 (0.16-1.16)	
Over-dominant	G/G-A/A	178 (62.7%)	37 (54.4%)	1	0.07
	A/G	106 (37.3%)	31 (45.6%)	0.60 (0.34-1.05)	
Log-additive	---	---	---	0.55 (0.35-0.84)	0.007

			rs11748222		
Model	Genotype	Controls	BPH	*OR (95% CI)	**p-Value
Co-dominant	T/T	221 (81%)	72 (84.7%)	1	0.28
	C/T	42 (15.4%)	8 (9.4%)	1.60 (0.71-3.63)	
	C/C	10 (3.7%)	5 (5.9%)	0.56 (0.18-1.76)	
Dominant	T/T	221 (81%)	72 (84.7%)	1	0.6
	C/T-C/C	52 (19.1%)	13 (15.3%)	1.20 (0.61-2.37)	
Recessive	T/T-C/T	263 (96.3%)	80 (94.1%)	1	0.28
	C/C	10 (3.7%)	5 (5.9%)	0.52 (0.17-1.65)	
Over-dominant	T/T-C/C	231 (84.6%)	77 (90.6%)	1	0.21
	C/T	42 (15.4%)	8 (9.4%)	1.65 (0.73-3.74)	
Log-additive	---	---	---	0.99 (0.60-1.64)	0.97

			rs1432982		
Model	Genotype	Controls	BPH	*OR (95% CI)	**p-Value
Co-dominant	A/A	175 (60.5%)	58 (69.9%)	1	0.44
	A/G	98 (33.9%)	22 (26.5%)	1.38 (0.78-2.42)	
	G/G	16 (5.5%)	3 (3.6%)	1.62 (0.45-5.89)	
Dominant	A/A	175 (60.5%)	58 (69.9%)	1	0.21
	A/G-G/G	114 (39.5%)	25 (30.1%)	1.41 (0.82-2.41)	
Recessive	A/A-A/G	273 (94.5%)	80 (96.4%)	1	0.55
	G/G	16 (5.5%)	3 (3.6%)	1.46 (0.41-5.27)	
Over-dominant	A/A-G/G	191 (66.1%)	61 (73.5%)	1	0.31
	A/G	98 (33.9%)	22 (26.5%)	1.33 (0.76-2.33)	
Log-additive	---	---	---	1.33 (0.85-2.10)	0.21

SNPStats was used to construct the haplotypes. *OR (95% CI): odd ratio and 95% confidential interval of SNP response to BPH when adjusted by age and WAA. **p-Value, adjusted p-value by age and WAA.

investigate rs10035432, rs11748222 and rs1432982 association with PCa and BPH risk. Our *in silico* functional analysis showed that TBP and c-EBPα transcriptional factors are present or absent depending on the given allele at their associated loci. We propose that these transcription factor changes can impact *SPINK1* gene expression, which may ultimately influence SPINK1's function in the etiology of BPH and PCa. In speculation, if TBP and c-EBPα were to bind to the promoter region, as a putative effect, *SPINK1*

will be expressed in order to regulate excessive amount of trypsin that may have secreted into the extracellular fluid. In theory, this mechanism of action could protect the prostate tissue against inflammation and, subsequently, prevent PCa development or an aggressive form of PCa. However, if TBP and c-EBPα lose their affinity to bind to the promoter region of *SPINK1* due to sequence changes in IL-6 RE, transcription of *SPINK1* may not be initiated and chronic inflammation will occur. This may lead to PCa

Table VI. *Haplotype frequencies and their association with high and low Gleason score (GS).*

	rs10035432	rs11748222	rs1432982	Total Frequencies	*Low GS	*High GS	**OR (95% CI)	*p*-Value
1	G	T	A	0.5038	0.4392	0.582	1	---
2	A	T	A	0.2004	0.2218	0.1712	1.44 (0.80-2.60)	0.22
3	G	T	G	0.168	0.2044	0.1211	2.01 (1.00-4.04)	0.05
4	G	C	A	0.0644	0.0682	0.0604	1.56 (0.66-3.70)	0.32
Rare	*	*	*	0.0634	NA	NA	1.11 (0.42-2.95)	0.83

*Low and high GS were defined as less advanced "if a case's GS is <7" and more advanced "if their GS is >7". **OR (95% CI): Odd ratio and 95% confidential interval. Global haplotype association *p*-value: 0.02.

Table VII. *Haplotype frequencies and their association with benign prostatic hyperplasia (BPH).*

	rs10035432	rs11748222	rs1432982	Total Frequencies	Controls	BPH	*OR (95% CI)	*p*-Value
1	G	T	A	0.5076	0.4896	0.5166	1	---
2	A	T	A	0.2282	0.2585	0.2175	0.63 (0.38 - 1.06)	0.08
3	G	T	G	0.1426	0.0947	0.1555	1.49 (0.71 - 3.14)	0.29
4	G	C	G	0.0599	0.0213	0.071	2.11 (0.63 - 7.00)	0.22
5	G	C	A	0.0381	0.0697	0.0279	0.46 (0.18 - 1.20)	0.12
6	A	C	A	0.0125	0.0526	0	0.64 (0.07 - 6.02)	0.70
7	A	T	G	0.0112	0.0136	0.0115	0.00 (0.00-NA)	1.00
Rare	*	*	*	0	0	0	1	---

SNPStats was used to construct the haplotypes. *OR (95% CI): Odd ratio and 95% confidential interval. NA, Not applicable. Global haplotype association *p*-value: 0.0043.

development and a more aggressive form of PCa. Further functional studies are required to validate the impact of TBP and c-EBPα on the regulation of *SPINK1* in BPH and PCa cells.

In this study, we found a possible positive association between rs1432982 and lower Gleason score in men of African descent. Previous studies have found that *SPINK1* is associated with high Gleason score (18, 45), while other studies reported an association with low Gleason score (24) or a null association (19, 22, 46, 47). Only one previous study has investigated SPINK1 and Gleason score in an African American cohort (24). In this previous study, AA men who were positive for SPINK1 protein over-expression were more likely to have a lower Gleason score compared to men negative for SPINK1 expression. We also found an inverse association between rs10035432 and BPH. We found that *SPINK1* was over-expressed in the BPH cell line (data not shown), which is consistent with previous findings (17). However, a previous study reported that SPINK1 expression was absent in benign prostate tissue (48). There is limited amount of data available about the role SPINK1 plays in BPH etiology. Thus, further investigation is needed to confirm SPINK1 expression in BPH cases. The regulation of *SPINK1* in prostate cancer cells is unclear.

A major strength of this study provides insight on genetic variations in the promoter region of *SPINK1*. However, we could have missed some associations, because we did not include SNPs across *SPINK1*, especially polymorphisms in the coding region. Also, we are the first to genotype rs11748222 and rs1432982 in controls, PCa and BPH who were African American. These SNPs were not reported as a part of the HapMap project. Given the modest size of our sample size and the limitation of other ethnic groups not included in this study, our findings warrant further validation in a lager cohort, which will include men from different ethnic backgrounds.

Conclusion

Our findings showed no association between *SPINK1* promoter variants and PCa risk. However, *SPINK1* promoter variants are likely to be associated with the risk of BPH.

Conflicts of Interest

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

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