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Interactive effect of TLR SNPs and exposure to sexually transmitted infections on prostate cancer risk in Jamaican men

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Abstract

Background: Prostate cancer (PC) risk increases with African ancestry and a history of sexually transmitted infections (STIs). Also, single-nucleotide polymorphisms (SNPs) in toll-like receptor (TLR) genes influence PC risk. This pilot study explores interactions between STIs and TLR-related SNPs in relation to PC risk among Jamaican men.

Methods: This case-control study evaluates two TLR related SNPs in 356 Jamaican men (194 controls and 162 cases) with or without history of STIs using stepwise penalized logistic regression in multivariable analyses.

Results: Age (odds ratio [OR] = 1.08; 95% confidence interval [CI]: 1.04–1>.12; $p < .001$) and IRF3_rs2304206 GG genotype (OR = 0.47; 95% CI: 0.29–0<.78; $p = .003$) modulated PC risk in people with history of STIs. In the population with no history of STIs, resulting interactions between risk factors did not survive correction for multiple hypothesis testing.

All author contributed equally to this work.

Conclusion: Overall, an interaction between the IFR3_rs2304206 variant and a history of exposure to STIs leads to greater decrease of PC risk than the presence of polymorphic genotype alone. These findings are suggestive and require further validation. Identification of gene variants along with detection of lifestyle behaviors may contribute to identification of men at a greater risk of PC development in the population.

KEYWORDS

black, prostate cancer, sexually transmitted infections, single nucleotide polymorphisms, StepPLR, toll-like receptor (TLR)

1 | INTRODUCTION

Prostate cancer (PC) is the most common cancer among men and the second leading cause of death in the United States.[1] While age, family history and race have been identified as significant risk factors for PC,[2] recent evidence suggests an increased PC risk associated with prior exposure to sexually transmitted infections (STIs).[3,4] It is suggested that the association between PC risk and STIs may be related to the exposure to pathogens, which causes chronic inflammation within the prostate and ultimately leads to enhanced cell proliferation and carcinogenesis.[5,6] In response to exposure to pathogens, the innate immune system serves as the first line of defense. Specifically, a conserved family of pattern recognition receptors, called the toll-like receptor (TLR) family, induces inflammatory cytokine and chemokine genes leading to microbial elimination.[7] A more recent report has demonstrated TLRs, specifically TLR subclass 4 (TLR4) and TLR9, may contribute to PC pathogenesis by stimulating prostate epithelial cell proliferation in response to pathogens.[8] Overall, TLR signaling cascades stimulate the expression of inflammatory chemokines, cytokines, and interferons, resulting in local inflammation. Genetic variations in these genes may lead to an imbalanced immune response (over exuberant or inadequate), resulting in excessive and harmful inflammation. Several TLR1-TLR4-TLR6-IL10 sequence variants are linked to an increase[9-11] and decrease[12] in PC risk. In addition, several epidemiological studies have found an increased risk of PC in association with history of prostatitis and STIs.[13-15] Other aspects of sexual behavior (e.g., number of sexual partners, condom usage, and age at first sex) have also been associated with PC risk,[3] as sexual activity may be a marker for higher risk of exposure to STIs which in turn have been linked to PC risk.

As men of African descent are at increased risk for inflammatory diseases,[16] genetic variations that lead to increased inflammatory responses in the presence of infectious agents may play an important role in disposing them to PC risk. Since TLRs play an important role in launching an inflammatory response against STIs, TLR associated genetic susceptibility modifies PC risk among men of African descent.[17,18] The current study seeks to evaluate interactions between TLR gene variants and STI exposure, in relation to PC risk among Jamaican men. Besides, very few address impact of TLR gene variants or exposure of STIs on PC risk among men of African descent even though the disparity in disease incidence is well known. In our previous study on TLR associated single-nucleotide polymorphisms (SNPs) and PC risk among US black men and Jamaican men, TLR6_rs2381289 and IRF3_rs2304206 were reported to be significantly associated with PC risk in Jamaican men.[18] However, these associations did not remain statistically significant after adjustment of multiple testing. Hence, we selected these two genetic markers from our previous study to further evaluate their role in modulating PC risk in Jamaican men with or without the history of STIs and using improved multivariable models with stepwise method based on penalized logistic regression (LR).

2 | METHODS

2.1 | Subjects

The current study included 356 DNA samples (163 cases and 195 controls) from a larger study of 515 Black men (243 cases and 275 controls). Details of the study population (case and control ascertainment, inclusion criteria) have been previously published elsewhere.[19] Briefly, patients were recruited from urology clinics at the two main tertiary hospitals and from private practitioners in the Kingston Metropolitan area in Jamaica. Based on clinical practice in Jamaica, the prevalence of PC with a prostate-specific antigen (PSA) of 3 ng/ml is as high as 15%. A single standard ultrasound-guided biopsy was performed on men attending the clinics for the first time during the recruitment period with abnormal DRE and PSA >3.0 μg/L. From the biopsy only, histologically-confirmed PC was graded according to the Gleason scoring system by a local uropathologist. Men who were biopsied and were PC free were excluded from study (i.e., were not used as controls). No biopsies were performed on the controls. Men on 5-alpha reductase inhibitors were also excluded from the study. Ethics approval was obtained from the University of the West Indies, Mona and Fox Chase Cancer Center Institutional Review Boards. Study participants provided written informed consent to participate in the study.

2.2 | Genotyping

TLR6_rs2381289 and IRF3_rs2304206 sequence variants were analyzed in this study. Of the 356 samples used the in analyses, genotype data of 216 samples was used as part of a collaborative project that is previously published.[18] Additional 141 samples were genotyped independently for this study. We randomly included several samples from the previously genotyped batch (216 subjects) in our new genotyping batch to cross check any variation which may arise due to change in platforms (batch effect). We obtained same genotypes for the random samples as was reported earlier, verifying that the change of genotyping platform did not lead to any variations in the results. Hence, the data from 216 subjects was merged with recently genotyped 141 subjects to results in a single dataset from 356 subjects. Quality controls, data management of genotype data, testing for Hardy–Weinberg equilibrium (HWE) are documented elsewhere.[18,20] An additional 141 individuals were genotyped for this study using TaqMan SNP genotyping assay. The experiments were performed using Applied Biosystems Step One Plus Real-Time PCR system (ThermoFisher Scientific/Life Technologies). The genotype calls were evaluated and analyzed via StepOne Software, version 2.3. SNPs were tested for HWE after addition of new genotype data.

2.3 | Epidemiologic and anthropometric data

A standardized interview administered questionnaire permitted collection of demographic, medical history, dietary habits, lifestyle factors and sexual behavior data. The data on physical activity was collected qualitatively using (i) leisure physical activity and (ii) nature of work. Participants were asked if they engaged in leisure physical activity (exercise or walking) for at least 20 min that makes them breathe heavier and heart beat faster (responses were no physical activity, fewer than once per week, one or two times per week or at least three or more times a week). Participants were also asked to describe the nature of their work, that is, mainly sitting, not much walking, a lot of walking, but not lifting or carrying heavy things, a lot of walking, often carrying things, or climbing stairs or going uphill and heavy physical work, such as carrying or lifting heavy things, digging and shoveling. Therefore, in our analysis using nature of work and measure of physical activity we categorized as follows: Inactive (no physical activity or mainly sitting work, not much walking), moderately inactive (fewer than once per week or quite a lot of walking, but not lifting or carrying heavy things), moderately active (one or two times per week or lot of walking, often carrying things, or climbing stairs or going uphill), and active (walking at least greater than three times a week or heavy physical work, carrying or lifting heavy things, digging, and shoveling). Smoking status (yes or no) was taken into account for both current and ex-smokers. Behavioral information included age at sexual intercourse initiation, lifetime sexual partners (number of partners during lifetime and within the past year), personal history of STIs, such as syphilis, gonorrhea, and genital sores (yes or no), prostitution (ever had sex with a commercial sex worker or prostitute) and condom use (frequency of use with regular partner and non-regular partners). Prostitution was a measure of whether a study participant ever had sex with a commercial sex worker or prostitute.

2.4 | Statistical analyses

Differences in demographic study participant characteristics between cases and controls were assessed using χ^2 statistics for categorical variables and the Wilcoxon Rank-Sum Test for continuous variables using the STATASE (13.0), StataCorp.[21]

The association between PC risk and TLR SNPs, expressed as odds ratios (ORs) and corresponding 95% confidence intervals (CIs), were estimated using LR models in STATA SE (13.0), both with and without adjustment for age. The analyses were run separately after stratifying for history of STIs. LR analyses for genetic variants and PC risk were conducted under codominant and additive genetic models using major or common genotype as the reference category.

Further, the interactions among genetic variants and risk factors were tested using multivariable models with stepwise method based on penalized logistic regression (StepPLR), as implemented in the StepPLR package in R.[22,23] Penalized LR stabilizes the coefficients in a LR model, by adding a penalty term to the likelihood function which results in shrunken coefficients. This method allows for efficient handling of multi-collinearity and sparse cells in multifactor contingency tables, which are typical problems encountered by regular LR when considering higher-order interactions. The regularization parameter λ[22] was chosen by running cross validation analyses with different values of λ using the StepPLR package, and the value of λ (=1) corresponding to the lowest value of Akaike information criterion was selected. In all multivariable models the following variables were included: age (as continuous variable), smoking status (yes or no), physical activity (inactive or active), number of sexual partners in a month (none, 1–3 per month, >4 per month), and use of condom with non-regular partners (never or always). StepPLR was performed for the total population as well as the study set after stratification by history of STIs. All p values (except in Table 1) were subjected to multiple hypothesis testing using the Benjamini–Hochberg (BH) false discovery rate (FDR)[24] and statistical significance was determined using FDR <0.05.

3 | RESULTS

3.1 | Study population characteristics

The characteristics for the study population in this analysis (163 cases and 195 controls) are summarized in Table 1. PC cases were older ($p < .0001$), had significantly higher PSA levels ($p < .0001$) and were more likely to have a family history of PC ($p = .027$) than controls. Controls reported higher attainment in secondary and higher

TABLE 1 Characteristics of study population by case or control status

Characteristics	Cases	Controls	*p* Value
Number of participants	163	195	
Age (years), median (range)	69 (49–80)	63 (40–80)	<.0001[a]
BMI (kg/m^2), mean (*SD*)	25.28 (4.41)	25.01 (4.06)	.2782
PSA (ng/ml), median (range)	33.75 (2.1–10,000)	1.8 (0.1–9.9)	<.0001[a]
Smoking s, n (%)			.007
No	41 (25.31)	74 (38.95)	
Yes	121 (74.69)	116 (61.05)	
Family history of PC, n (%)			.027
No	132 (80.98)	174 (89.23)	
Yes	31 (19.02)	21 (10.77)	
Physical activity, n (%)			
Inactive	31 (19.14)	41 (21.35)	.028
Moderately inactive	32 (19.75)	52 (27.08)	
Moderately active	62 (38.27)	77 (40.10)	
Active	37 (22.84)	22 (11.46)	
Education, n (%)			.016
Primary or less	147 (91.3)	155 (81.15)	
Secondary	8 (4.97)	26 (13.61)	
Tertiary	6 (3.73)	10 (5.24)	
Marital status, n (%)			.281
Married	100 (61.73)	125 (65.10)	
Previously married	32 (19.75)	25 (13.02)	
Others	30 (18.52)	42 (21.35)	
Prostitution (N = 440), n(%)			.778
No	126 (82.35)	147 (83.52)	
Yes	27 (17.65)	29 (16.48)	
Number of sexual partners (N = 447), n(%)			.103
None	72 (46.15)	67 (37.43)	
1–3/month	49 (31.41)	54 (30.17)	
>4/month	35 (22.44)	58 (32.4)	
Condom use with regular partners			.468
Never	117 (73.13)	132 (68.75)	
Always	43 (26.88)	59 (30.73)	
Condom use with non regular partners			.223
Never	86 (53.42)	83 (43.23)	
Always	74 (45.96)	106 (55.21)	
STI history (N = 499) n (%)			.115
No	59 (36.65)	85 (44.97)	
Yes	102 (63.35)	104 (55.03)	
Gleason score, n (%)			
3	1 (0.61)		
6	65 (39.88)		
7	56 (34.36)		
8	18 (11.04)		
9	16 (9.82)		
10	2 (1.23)		
Missing	5 (3.07)		

Abbreviations: BMI, body mass index; PC, prostate cancer; PSA, prostate-specific antigen; STI, sexually transmitted infection.

[a]Wilcoxon sum rank test.

education, were less physically active and were more likely to be reported as non-smokers ($p = .007$) than cases. Cases and controls showed similar mean BMI ($p = .2782$). With respect to sexual behavior, usage of condoms with regular ($p = .468$) and non-regular partners ($p = .223$) was not significantly different between cases and controls. Prostitution, history of STIs, number of sexual partners and marital status were not found to be significantly different between cases and controls.

The minor allele frequency for TLR-associated SNPs, TLR6_ rs2381289 and IRF3_rs2304206, among Jamaican men was found to be 0.23 and 0.32, respectively. Both SNPs followed HWE before and after the addition of samples in this study.

3.2 | Association between TLR-associated sequence variants and PCA risk

IRF3_rs2304206 and TLR6_ rs2381289 were associated with PC risk for unadjusted and age-adjusted LR models as shown in Table 2. In particular, the presence of IRF3_rs2304206 GG genotype was associated with an approximately 60% reduction in PC risk (OR = 0.40; CI: 0.19–0.81; $p = .012$). This association was true for age-adjusted models as well (OR = 0.36, CI: 0.17–0.76; $p = .008$). Also presence of TLR6_ rs2381289 GA genotype was associated with 1.7–1.9 fold increase in PC risk under unadjusted ($p = .014$, CI: 1.11–2.67) and age-adjusted ($p = .009$, CI: 1.16–2.96) LR models. Under the additive genetic model the IRF3_rs2304206 sequence variant allele was associated with reduced (~ 70%) PC risk ($p = .027$, CI: 0.52–0.96); whereas, TLR6_ rs2381289 did not remain significant under this model. After stratification of the study population by history of STIs (Table 3), IRF3_rs2304206 (GG) was found to be significant in the population with a history of STI ($p = .008$, CI: 0.10–0.76) and had lower ORs (0.24) when compared with the total population. However, the inheritance of TLR6_ rs2381289 (GA) genotype was associated with elevated PC risk (OR = 1.85) but this association did not survive multiple-testing correction. Hence, further interaction analyses were

TABLE 2 TLR SNPs and their association with prostate cancer risk in total population

Total population											
SNP	Genotypes	Cases	Controls	OR unadjusted	CI (95%)	*p* Unadjusted	FDR[a]	OR age-adjusted	CI (95%)	*p* Age-adjusted	FDR[a]
Irf3_rs2304206	AA	85	87	1 (ref.)				1 (ref.)			
	AG	65	75	0.88	0.56–1.38	.599	0.599	0.87	0.54–1.40	.579	0.579
	GG	13	33	0.40	0.19–0.81	**.012**	**0.036**	0.36	0.17–0.76	**.008**	**0.024**
	GG + AG	78	108	0.70	0.52–0.96	**.027**	**0.041**	0.67	0.49–0.93	**.018**	**0.027**
TLR6_rs2381289	GG	82	121	1 (ref.)				1 (ref.)			
	GA	75	64	1.72	1.11–2.67	**.014**	**0.042**	1.86	1.16–2.96	**.009**	**0.027**
	AA	5	10	0.73	0.24–2.23	.591	0.591	0.78	0.24–2.49	.681	0.681
	AA + GA	80	74	1.32	0.92–1.90	.12	0.18	1.39	0.95–2.05	.088	0.132

Note: Bold values are statistically significant. Allele counts for Irf3_rs2304206 in total population: A: cases-235, controls-249; G: cases-91, controls-141. Allele counts for TLR6_rs2381289 in total population: A: cases-85, controls-84; G: cases-239, controls-306.

Abbreviations: CI, confidence interval; FDR, false discovery rate; OR, odds ratio; ref, reference; SNP, single-nucleotide polymorphism; TLR, toll-like receptor.

[a]Statistically significant associations were determined using FDR <0.05.

carried out using IRF3_rs2304206 to test gene-environment interactions using StepPLR.

3.2.1 | StepPLR analyses

The results of multivariable models with age, smoking status, physical activity, condom usage, number of sexual partners and genetic marker IRF3_rs2304206, analyzed using StepPLR for the total population as well as the populations stratified by history of STIs are summarized in Table 4. This method produced results in terms of main effects and interactive effects of tested SNP locus and other factors in association with PC risk. In the total population, after BH correction, age (OR = 1.04; 95% CI: 1.02–1.06; FDR = 0.005), genetic variant IRF3_rs2304206 (GG) (OR = 0.58; 95% CI: 0.38–0.87; FDR = 0.027) and the interaction of physical activity with age (OR = 1.03, 95% CI: 1.01.04, FDR = 0.005) were found to be associated with PC risk. Among individuals with a history of STIs, age (OR = 1.08; 95% CI: 1.04–1.12; FDR = 0.012) and possession of the IRF3_rs2304206 (GG) genotype (OR = 0.47; 95% CI: 0.29–0.78; FDR = 0.018) were associated with PC risk. In the population with no history of STIs (Table S1), a number of interactions were significantly associated with PC risk, however, these were no longer significant after adjusting for multiple testing.

4 | DISCUSSION

TLRs play critical roles in the reproductive tract by signaling the presence of infection or other inflammatory stimuli,[25] and prostatic inflammation or response to infection may have a direct effect on the

TABLE 3 TLR SNPs and their association with Prostate cancer risk in population stratified by history of STIs

Pop with STI hist.								Pop with No STI hist.					
SNP	Genotypes	Cases	Controls	OR	CI (95%)	*p*	FDR[a]	OR	Cases	Controls	CI (95%)	*p*	FDR[a]
Irf3_rs2304206	AA	54	48	1 (ref.)				1 (ref.)	30	39			
	AG	42	37	1.03	0.56–1.81	.925	0.925	0.89	22	32	0.43–1.83	.76	0.434
	GG	6	19	0.24	0.10–0.76	**.008**	**0.024**	0.65	7	14	0.23–1.81	.41	0.434
	GG + AG	48	56	0.63	0.41–0.97	.037	0.055	0.78	29	46	0.47–1.28	.33	0.434
LR6_rs2381289	GG	45	61	1 (ref.)				1 (ref.)	36	55			
	GA	52	38	1.85	1.05–3.27	.033	0.099	1.4	23	25	0.69–2.84	.34	0.51
	AA	4	5	1.08	0.27–4.2	.908	0.908	0.3	1	5	0.34–2.72	.28	0.51
	AA + GA	56	43	1.49	0.92–2.39	.11	0.165	1.1	24	30	0.56–1.81	.75	0.75

Note: Bold values are statistically significant. Allele counts for Irf3_rs2304206 in population with a history of STI: A: cases-150, controls-133; G: cases-54, controls-75. Allele counts for Irf3_rs2304206 in population without a history of STI: A: cases-82, controls-110; G: cases-36, controls-60. Allele counts for TLR6_rs2381289 in population with a history of STI: A: cases-60, controls-48; G: cases-142, controls-160. Allele counts for TLR6_rs2381289 in population without a history of STI: A: cases-25, controls-35; G: cases-95, controls-135.

Abbreviations: CI, confidence interval; FDR, false discovery rate; OR, odds ratio; ref, reference; SNP, single-nucleotide polymorphism; STI, sexually transmitted infection; TLR, toll-like receptor.

[a]Statistically significant associations were determined using FDR <0.05.

TABLE 4 Association and Interactions between SNP *Irf3_rs2304206*/Life style factors and prostate cancer risk in population with history of STIs and total population

Population size	Main effects or gene	Genotype	Cases	Controls	Interactions	OR	CI (95%)	*p*	FDR
Total population[a]									
	Age					1.04	1.02–1.06	**<.001**	**0.005**
	Irf3_rs2304206	AA	85	87		1.42	1.0–1.94	.025	0.052
	Irf3_rs2304206	AG	65	75		1.22	0.88–1.68	.227	0.284
	Irf3_rs2304206	**GG**	13	33		0.58	0.38–0.87	**.008**	**0.027**
					Physically active: Age	1.03	1.01–1.04	**<.001**	**0.005**
					Physically inactive: Age	1.01	1–1.03	.02	0.055
	No Smoke					0.82	0.65–1.05	.116	0.166
	Smoke					1.21	0.95–1.55	.116	0.166
	Physically active					0.83	0.55–1.26	.384	0.384
	Physically inactive					1.2	0.79–1.83	.384	0.384
Population with history of STIs[b]									
	Age					1.08	1.04–1.12	**<.001**	**0.012**
	Irf3_rs2304206	AA	54	48		1.57	1.07–2.31	.023	0.069
	Irf3_rs2304206	AG	42	37		1.35	0.9–2.01	.145	0.29
	Irf3_rs2304206	**GG**	06	19		0.47	0.29–0.78	**.003**	**0.018**
	Physically active					1.07	0.85–1.34	562	0.613
	Physically inactive					0.94	0.75–1.17	.562	0.613
					NSP. none: Physically active	1.06	0.67–1.66	.803	0.803
					NSP. none: Physically inactive	0.67	0.41–1.11	.117	0.281
					NSP. 1–3/m: Physically active	1.29	0.79–2.1	.307	0.432
					NSP. 1–3/m: Physically inactive	0.71	0.43–1.17	.176	0.302
					NSP. >4/m: Physically active	0.78	0.48–1.27	.324	0.432
					NSP. >4/m: Physically inactive	1.98	1.16–3.38	.013	0.052

Note: Bold values are statistically significant.

Abreviations: CI, confidence interval; FDR, false discovery rate; OR, odds ratio; STI, sexually transmitted infection.

[a]Statistically significant associations were determined using FDR <0.05 + allele counts forIrf3_rs2304206 in total population: A: cases: 235, controls: 249; G: cases: 91, controls: 141.

[b]Allele counts for Irf3_rs2304206 in population with history of STIs: A: cases: 150; controls: 133; G: cases: 54, controls: 75.

onset of PC. This is the first study to evaluate the effect of interaction between STIs and TLR SNPs on PC risk among men of African descent. StepPLR analyses resulted in many gene-environment interactions; however, the discussion will be limited to only statistically significant results.

In a hospital-based case-control study of Jamaican black men we observed that tested SNPs in TLR6 and IRF3 genes may contribute to PC risk and this association is modified by previous exposure to STIs status. Under univariate unadjusted LR models, possession of IRF3_rs2304206 GG genotype was inversely associated (OR = 0.40; CI: 0.19–0.81) with PC risk whereas TLR6_ rs2381289 GA genotype increased the risk of developing PC (OR = 1.7; CI: 1.11–2.67). To further evaluate the effect of STIs exposure on PC risk the population was stratified by history of ever/never exposure to STIs. Among individuals exposed to STIs, the TLR6_ rs2381289 variant did not remain significant after multiple testing corrections. Hence, we proceeded with IRF3_rs2304206 for further multivariable LR analysis using StepPLR method where we found older age and the IRF3_rs2304206 GG genotype were individually linked to PC risk in both the overall population and in the stratified population with history of STIs exposure. Decreased risk towards PC upon possession of variant alleleIRF3_rs2304206 indicates disease protecting nature of the polymorphism in Jamaican men. Interferon regulatory factor 3 (IRF3) is a transcriptional factor that plays a crucial role in the activation of innate immunity and inflammation in response to microbial infection and also has been suggested as a potential tumor suppressor gene.[26] SNP rs2304206 has been reported as a significant expression quantitative trait loci eQTL (http://www.gtexportal.org/), which means the alternative alleles of rs2304206 are associated with different levels of IRF3 expression. In addition, rs2304206 falls in the regulatory region of the gene[27] and genotype AA has been linked to lower IRF3 expression in blood cells among the Japanese subgroup.[28] Inverse relationship with PC in our study puts forward GG genotype of the SNP may play a role in the expression of IRF3 in a way that it aids/retains tumor suppressing nature. Further, a study looking into the role of IRF3 in glioma invasive properties suggested that in vitro adenovirus mediated IRF3 activation resulted in upregulation of IRF3 thus inhibiting glioma proliferation, migration and invasion.[29]

This study further concluded that agents that promote IRF3 activation and expression (adenovirus in this case) may serve as potential target therapy against glioma. In our study, we see a statistically significant decrease in the ORs from 0.58 (in the total population, $p = .008$, FDR = 0.027) to 0.47 (in the sub-population with STI exposure, $p = .003$, FDR = 0.018) which may suggest (on the basis of the above study) that, in addition to the possession of variant genotype, microbial agents from STIs exposure activate IRF3 gene assisting its tumor suppressing behavior. Additionally, the protective response of IRF3 against viral infections has been shown in other studies, which further supports this account.[30,31] In a previous study, after adjustment for multiple hypothesis testing, we did not observe a statistically significant protective association of IFR3_rs2304206 with PC.[18] However in this study we now report that a combination of the polymorphic genotype of IFR3_rs2304206 and a history of exposure to STIs provides added shielding from PC as compared with possession of polymorphic genotype alone. This association remained statistically significant after adjustment for multiple hypothesis testing.

In addition to age and variant genotype of IRF3 gene, interaction between age and physical activity was observed as a factor modestly associated with PC risk in the total population. Although physical activity is recommended as primary prevention in PC, studies have reported an inverse relationship between the level of physical activity and PC risk.[32,33] Physical activity can have different influences on carcinogenesis, depending on energy supply and age of the subject. This notion is indirectly supported by studies that show intense physical activity may activate cellular stress signaling, accumulation of large amounts of reactive oxygen species which can lead to DNA damage, or may support tumorigenesis by triggering proinflammatory signaling.[34]

LR is the most commonly used tool to find correlation between risk factors and disease risk, but in the case of SNP data with two or three levels of genotype factors along with other confounding variables, problems of overfitting models may arise especially if data size is small.[22] PLR is well suited for modeling a large number of variables and sample size does not limit the number of such variables.[35] We realize sufficiently powered studies of interactions need large sample sizes. Hence, step-wise PLR approach was used as quadratic penalization yields a stable fit, even with a large number of parameters and small data size, thus making it possible to use LR in building interaction models. StepPLR has been demonstrated to have excellent power to identify covariate effects as well as superior prediction accuracy compared with competing methods like Classification and Regression Tree Analysis and Multifactor Dimensionality Reduction.[35] These results were further filtered through multiple hypothesis testing procedures. Hence the overall conclusion of a protective effect of IRF3_rs2304206 on PC remains unchanged in Jamaican men after addition of ~140 new samples to the study.

It is interesting to note that the Jamaica Health and Lifestyle Study II (JHLS II) which is a survey of a nationally representative sample in Jamaica, reported a STI prevalence in men of 18.2% (95% CI, 15.4%–21.3%).[36] The prevalence of a history of STIs in our study was significantly higher than what has been reported in Jamaica and may reflect a bias due to recruitment of the study sample from the hospital and urologist practices. One of our co-authors who evaluates patients in the urology clinic reports that the five most common problems seen in urology clinics are PC, BPH, stones, urinary tract infections and bladder cancer. They also treat patients with urethral stricture disease which is causally related to STIs (gonococcal and chlamydial urethritis). Therefore, we were not surprised to see a higher prevalence of STI among our controls than in the general population. Furthermore, based on our data on education attainment, there is lower representation of men from higher SES categories (i.e., men with tertiary education) which may also account for this high prevalence of STI infection. Nevertheless, our analyses also speculate the role of STIs in addition to genetic polymorphisms in the susceptibility to PC by modulating immune response. Identification of gene variants as the inherited factor of PC risk along with detection of lifestyle behaviors may contribute to development of better diagnostic strategies and accurate identification of men in the population at a greater risk of PC development. We plan to take the results of this exploratory analysis further using molecular biological studies and bioinformatics analyses to confirm the role of IRF3_ rs2304206 as an eQTL with respect to PC and STIs which could be explored for both PC therapy and identifying target groups at higher risk. In addition, it would be important to investigate whether our study findings are unique to Jamaican men or also observed in non-Jamaican men of African descent or European men. For example, the evaluation of whether innate immune sequence variants combined with STIs may modify PC could be conducted by leveraging on existing studies, such as the cancer genetic epidemiology markers and the prostate, l ung, colorectal and ovarian cancer screening trial (following the necessary approvals). Studies involving Jamaican and non-Jamaican men can also be performed by leveraging on existing collaborative networks within our consortium, the African Caribbean Cancer Consortium and also through a strong alliance with the Prostate Cancer Transatlantic Consortium. These comparative studies are in the planning phase.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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