Complex Interactions among Toll-Like Receptor Related Sequence Variants and Prostate Cancer Susceptibility among men of African Descent



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INTRODUCTION

- > In 2018, 164,690 men will have new prostate cancer (PCa) cases in the United States.
- Men with localized/regional PCa have a 5-yr survival rate following treatment. However, once the cancer spreads, the 5-yr survival rate drops down to 30%.
- African-American men are 1.6 fold more likely to receive a PCa Diagnosis and twice more likely to die from the disease than European-Americans
- > Two independent studies suggest PCa health disparities may be attributed to dysregulations in immune and inflammatory circuits.
- > Toll-Like Receptors (TLR) are the gate keepers and regulators of innate immune responses to harmful pathogens.
- Genetic alterations in TLRs may increase cytokine/chemokine production, cell proliferation, cell differentiation and cell survival, which may ultimately cause an increase in PCa susceptibility.
- > To date, there have been six published reports that show inheritance of TLR sequence variants alter cancer outcomes.

Figure 1: Toll-Like Receptor Signaling Pathway

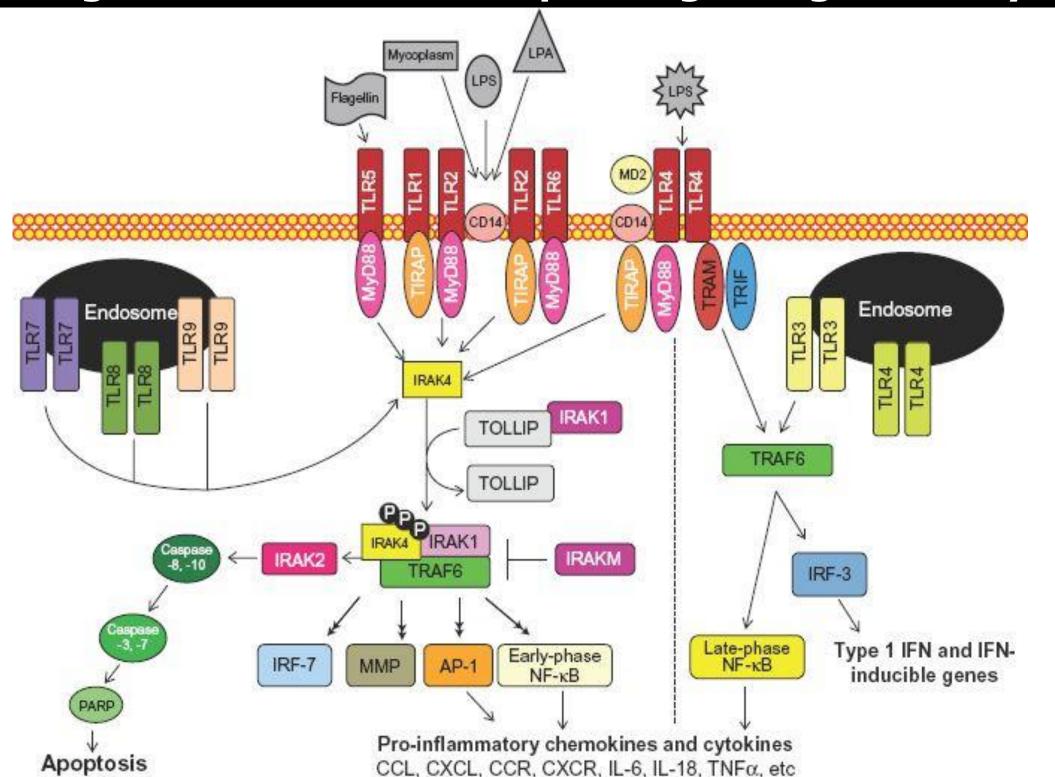


Table 4. TID a	ene-gene interactions in literat	
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IGDIC TI IEN C		

Gene	Case/Control	Disease	Reference
IRAK4 TLR1	1,383/780	Prostate Cancer	Sun, 2006
TLR2 IRAK4	279/535	Prostate Cancer	Rogers et.al, 2013
TLR2 TLR4 TLR9	420/842	Cervical Cancer	Jin et. al, 2017
TLR10 TLR6 IRF3	279/535	Prostate Cancer	Rogers et.al, 2013
NFKB1 TLR4	915/1719	Colorectal Cancer	Kopp, 2015
TLR2 CD14	93/104	Oral Squamous Cell Carcinomas	Zeljic et.al, 2014

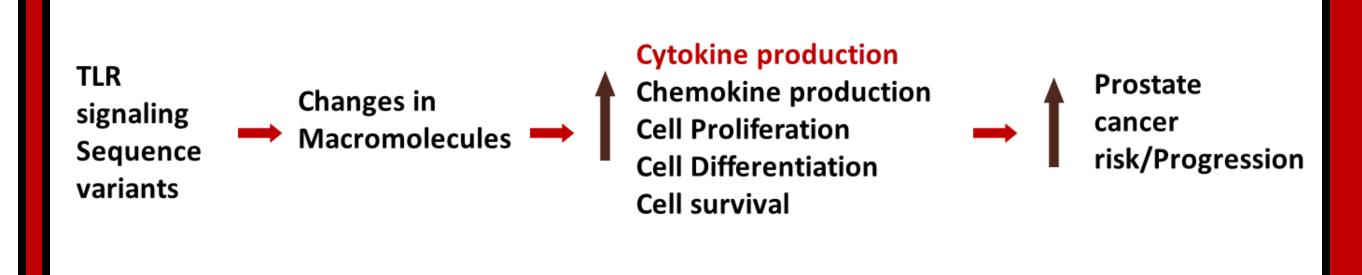
RESEARCH GAP

There are limited studies that focus on the impact of two sequence variants in toll-like receptor associated genes that jointly modify PCa risk among men of African descent.

RESEARCH OBJECTIVE

Evaluate the joint modifying effects of 465 single nucleotide polymorphism (SNP) pairs in relation to PCa among men of African descent.

HYPOTHESIS



STUDY DESIGN

- ❖ The project was performed using a case/control study design.
- ❖ 814 men (603 U.S men and 211 Jamaican men) were recruited from cancer screening programs, hospitals, or cancer centers located in Washington, D.C., South Carolina, and Kingston, Jamaica.
 - > U.S. population: 170 cases, 433 controls
- > Jamaican population: 109 cases, 102 controls

METHODS

- Assess differences in the genotype frequency comparing cases and controls using Chi-square and Fisher's Exact Test
- **Logistic Regression Analysis**
- > Calculated risk estimates and corresponding 95% Confidence Intervals
- Adjusted for potential confounders [age, Prostate-Specific Antigen (PSA) and West African Ancestry (WAA)]
- PLINK & Statistical Analysis Software (SAS) 9.4
- Genome association analysis toolset http://zzz.bwh.harvard.edu/plink/
- Analyzed 465 pair-wise interactions and provided the interactions considered to be statistically significant (p<0.0001).</p>

CLINICAL RELEVANCE

Table 2. TLRs used in Current Clinical Trials						
Gene	Treatment	Disease	Sponsor	Status	Trial Number	
TLR3	Bevacizumab Poly-ICLC Keyhole limpet hemocyanin	Glioblastoma Glioma	New York School of Medicine	Phase II	NCT02754362	
TLR7	Imiquimod	Breast Cancer Breast Neoplasm	New York School of Medicine	Phase II	NCT00899574	
TLR3	OC-L/Montanide ISA 51 VG Ampligen Prevnar	Ovarian Cancer Fallopian Tube Cancer Primary Peritoneal Cancer	Abramson Cancer Center of the University of Pennsylvania	Phase I Phase II	NCT01312389	
TLR7	852A	Breast Cancer Ovarian Cancer Endometrial Cancer Cervical Cancer	Masonic Cancer Center, University of Minnesota Pfizer	Completed	NCT00319748	

RESULTS

WT/VAR

Table 3. Role of TLR sequence variants in PCa among men of African descent

			WT/VAR			
			VS	Dominant	Recessive	
	Nucleotide		WT/WT			
Gene_dbSNP ID	Change	Function	P value	P-value	P-value	P-Trend
TOLLIP_rs5743899	T>C	T	0.018		0.945	
IRAK4_rs4251473	A>C	Intron 5	0.076	0.042	0.233	
TLR6_rs2381289	G>A	Testero o	0.052	0.057	0.940	0.102
IRAK4_rs4251520	C>T	Intron 9	0.075	0.083	0.927	0.136
TLR1_rs5743604	G>A	Intron 3	0.035	0.088	0.459	
IRAK4_rs4251545	A>G	Splicing(ESE/ ESS)	0.103	0.096	0.720	0.149
		nsSNP				
		benign				
TLR6_rs5743810	G>A	nsSNP	0.201	0.137	0.373	0.119
	O, 21	benign	0.202	01_02		0122
TLR6_rs5743818	C>A		0.146	0.176	0.801	0.241
TOLLIP_rs3168046	G>A	miRNA	0.925	0.191	0.069	0.062
IRAK4_rs4251467	C>T	Intron 3	0.314	0.219	0.269	0.164
TLR1_rs4833095	C>T	nsSNP	0.341	0.246	0.401	0.205
		benign				
TLR6_rs1039559	G>A	TFBS	0.405	0.272	0.261	0.187
TLR2_rs1898830	A>G	Intron 1	0.268	0.296	0.914	0.374
TLR4_rs11536887	A>G	miRNA	0.451	0.309	0.233	
TLR1_rs4624663	T>C	3' UTR	0.363	0.355	0.868	0.360
TLR4_rs1927906	T>C	Near Gene-3	0.270	0.356	0.699	0.697
TLR2_rs3804099	C>T	Exon 1	0.432	0.374	0.648	
TLR1_rs3923647	T>A	nsSNP	0.431	0.394	0.985	0.365
		possibly				
TOLLID ===================================	C \ A	damaging	0.410	0.207	0.704	0.410
TOLLIP_rs5743867	G>A	Calicina	0.419	0.397	0.794	
TLR4_rs5030710	C>T	Splicing (ESE/ESS)	0.381	0.412	0.933	0.492
TLR4 rs1927911	G>A	Intron 1	0.321	0.450	0.577	0.973
TLR10_rs10776483	G>A	Incion I	0.576	0.488	0.558	0.426
TLR10_rs11096955	T>C	nsSNP	0.676	0.622	0.756	0.618
	170	benign	01070	0.022	0.750	0.010
		Splicing(ESE/				
IRF3_rs2304206	G>A	ESS)	0.789	0.634	0.477	0.481
TLR10_rs11096957	T>G	nsSNP	0.631	0.634	0.952	
		possibly				
		damaging				
TLR1_rs5743595	G>A	Intron 2	0.818	0.703	0.987	0.603
TLR4_rs2149356	T>G	Intron 3	0.529	0.745	0.331	0.921
TLR10_rs4274855	T>C		0.667	0.792	0.987	0.925
TLR10_rs11466640	G>A		0.919	0.806	0.987	0.693
TLR2_rs7656411	G>T	Near Gene-3	0.771	0.808	0.929	0.906
TLR4_rs4986791	C>T	nsSNP	0.836	0.868	0.989	0.902
		possibly				
		damaging	<u> </u>		<u> </u>	• • • •
TLR6_rs3821985	G>C		0.724	0.903	0.152	0.427

Table 4. Two-way Interactions among TLR Related Sequence Variants among men of African descent using a Multiplicative Scale

		Overall Unadj.	Dominant Unadj.	Overall Age Adj.	Dominant Age Adj.
Gene_dbSNP ID	Gene_dbSNP ID2	P-value	P-value	P-value	P-value
TLR2_rs3804099	TLR10_rs11466640	0.0253	0.0172	0.0095	0.0083
IRAK4_rs4251520	TOLLIP_rs3168046	0.0146	0.0097	0.0101	0.0086
TLR10_rs11466640	TLR1_rs5743604	0.0026	0.0039	0.0081	0.0160
TLR2_rs7656411	TLR4_rs4986791	0.0201	0.0174	0.0230	0.0207
IRF3_rs2304206	TLR6_rs5743818	0.0056	0.0093	0.0110	0.0240
TLR1_rs4833095	TLR2_rs7656411	0.0771	0.0573	0.0444	0.0385
TLR2_rs1898830	TLR4_rs4986791	0.0319	0.0252	0.0507	0.0437
TLR10_rs4274855	TLR1_rs5743604	0.0094	0.0159	0.0130	0.0487
TLR6_rs5743810	TLR4_rs5030710	0.0556	0.0327	0.0663	0.0543
TLR6_rs1039559	IRAK4_rs4251467	0.0221	0.0152	0.0716	0.0586
TLR1_rs5743595	TLR1_rs5743604	0.0271	0.0515	0.0291	0.0608
TLR1_rs4624663	TOLLIP_rs3168046	0.0074	0.0247	0.0502	0.0741
TOLLIP_rs5743867	TLR10_rs11096957	0.0596	0.0667	0.5037	0.1326
TLR1_rs3923647	TOLLIP_rs3168046	0.0133	0.0279	0.1004	0.1606
IRAK4_rs4251545	TLR4_rs1927906	0.0027	0.0753	0.0447	0.1946
TLR4_rs1927911	IRAK4_rs4251520	0.0296	0.0719	0.1346	0.2486
TLR10_rs10776483	TLR4_rs5030710	0.0090	0.0296	0.0951	0.2763
TLR6_rs2381289	TLR2_rs3804099	0.0788	0.2801	0.3466	0.3184
TLR4_rs2149356	TLR6_rs5743818	0.0739	0.3917	0.0557	0.3416
TLR10_rs11096957	TOLLIP_rs5743899	0.0152	0.0794	0.4533	0.3721
IRAK4_rs4251473	TLR4_rs1927906	0.0029	0.1015	0.0573	0.3875
TLR6_rs3821985	TLR6_rs2381289	0.1032	0.1924	0.3130	0.6975

Table 5. Role of TLR sequence variant interactions in PCa among men of African descent using an Additive Scale

IRAK4_rs4251520	men of Africa	n descent using an Ad	uitive Stale		
TLR4 rs1927911					
IRAK4_rs4251520			-	P-value	
2 1.94 (0.94, 4.01) 0.026	_				0.321
3-4 1.70 (0.77, 3.77) 0.130	IRAK4_rs4251520		, ,		
≥1 var for one SNP			, ,		
TLR10_rs1076483			, , ,		
TLR10_rs10776483		≥1 var for one SNP	1.68 (0.83, 3.41)	0.041	
TLR10_rs10776483		≥1 variant allele for both SNPs	2.09 (0.99, 4.39)	0.035	
TLR4_rs5030710 1 1.10 (0.75, 1.61) 0.085 2 1.23 (0.70, 2.15) 0.423 3-4 0.24 (0.05, 1.25) 0.043 TLR4_rs2149356 0 1.00 (referent) 0.169 TLR6_rs5743818 1 0.98 (0.68, 1.43) 0.607 TLR10_rs4274855 0 1.00 (referent) 0.364 TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 2 0.92 (0.57, 1.49) 0.497 3-4 <0.001 (<0.001, >999.999) 0.978 ≥1 var for both SNPs 0.28 (0.07, 1.07) 0.034 TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776	TLR10 rs10776483				0.961
2 1.23 (0.70, 2.15) 0.423	_	1	,	0.085	
TLR4_rs2149356 0 1.00 (referent) 0.169 TLR6_rs5743818 1 0.98 (0.68, 1.43) 0.607 1 0.92 (0.62 (0.33, 1.19) 0.035 3-4 1.41 (0.34, 5.80) 0.594 TLR10_rs4274855 0 1.00 (referent) 0.364 TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 3-4 <0.001 (<0.001, >999.999) 0.978 ≥1 var for one SNP 0.82 (0.59, 1.20) 0.597 ≥1 variant allele for both SNPs 0.24 (0.06, 0.96) 0.029 E1 var for both SNPs 0.28 (0.07, 1.07) 0.034 TLR10_rs11466640 0 1.00 (referent) 0.459 TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776 RR1_rs5743604 1 0.87 (0.59, 1.27) 0.801 ≥1 var for both SNPs 0.27 (0.07, 1.03) 0.033		2	1.23 (0.70, 2.15)	0.423	
TLR6_rs5743818 1 0.98 (0.68, 1.43) 0.607 LR10_rs4274855 0 1.00 (referent) 0.364 TLR10_rs5743604 1 0.746 (0.495, 1.24) 0.532 2 0.92 (0.57, 1.49) 0.497 3-4 <0.001 (<0.001, >999.999) 0.978 ≥1 var for one SNP 0.82 (0.59, 1.20) 0.597 ≥1 variant alkele for both SNPs 0.28 (0.07, 1.07) 0.034 TLR10_rs11466640 0 1.00 (referent) 0.459 TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776 21 var for one SNP 0.83 (0.55, 1.44) 0.496 3-4 <0.001 (<0.001, >999.999) 0.981 E1 var for one SNP 0.87 (0.59, 1.27) 0.003 E1 var for one SNP 0.87 (0.59, 1.27) 0.801 ≥1 var for both SNPs 0.27 (0.07, 1.03) 0.033 E1 var for both SNPs 0.30 (0.08, 1.11) 0.034 TLR6_rs1039559 0 1.00 (referent) 0.213 IRAK4_rs4251467 1 0.96 (0.66, 1.39) 0.515 21 var for		3-4	0.24 (0.05, 1.25)	0.043	
2	TLR4_rs2149356	0	1.00 (referent)		0.169
TLR10_rs4274855	TLR6_rs5743818	1	0.98 (0.68, 1.43)	0.607	
TLR10_rs2743654 0 1.00 (referent) 0.364 TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 2 0.92 (0.57, 1.49) 0.497 3-4 < 0.001 (<0.001, >999.999) 0.978 ≥1 var for one SNP 0.82 (0.59, 1.20) 0.557 ≥1 variant allele for both SNPs 0.24 (0.06, 0.96) 0.029 1 var for both SNPs 0.28 (0.07, 1.07) 0.034 1 var for both SNPs 0.28 (0.07, 1.07) 0.034 1 var for both SNPs 0.28 (0.07, 1.07) 0.034 1 var for both SNPs 0.28 (0.07, 1.07) 0.034 1 var for both SNPs 0.28 (0.07, 1.07) 0.034 1 var for both SNPs 0.28 (0.07, 1.07) 0.045 2 var for both SNPs 0.87 (0.59, 1.27) 0.801 ≥1 var for both SNPs 0.27 (0.07, 1.03) 0.033 21 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 LR6_rs1039559 0 1.00 (referent) 0.213 1 RAK4_rs4251467 1 0.96 (0.66, 1.39) 0.515 2 var for both SNPs 0.20 (0.05, 0.77)		2	0.62 (0.33, 1.19)	0.035	
TLR1_rs5743604 1 0.746 (0.495, 1.124) 0.532 2 0.92 (0.57, 1.49) 0.497 3-4 <0.001 (<0.001, >999.999) 0.978 ≥1 var for one SNP 0.82 (0.59, 1.20) 0.597 ≥1 variant allele for both SNPs 0.24 (0.06, 0.96) 0.029 TLR10_rs11466640 0 1.00 (referent) 0.459 TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776 2 0.893 (0.55, 1.44) 0.496 3-4 <0.001 (<0.001, >999.999) 0.981 ≥1 var for both SNPs 0.27 (0.07, 1.03) 0.033 ≥1 variant allele for both SNPs 0.27 (0.07, 1.03) 0.033 1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 var for both SNPs 0.90 (0.66, 1.39) 0.515 2 var for both SNPs 0.91 (0.64, 1.31) 0.721 ≥1 var for both SNPs 0.20 (0.05, 0.77)		3-4	1.41 (0.34, 5.80)	0.594	
2 0.92 (0.57, 1.49) 0.497 3-4 <0.001 (<0.001, >999.999) 0.978 ≥1 var for one SNP 0.82 (0.59, 1.20) 0.597 ≥1 variant allele for both SNPs 0.24 (0.06, 0.96) 0.029 ≥1 var for both SNPs 0.28 (0.07, 1.07) 0.034 ∑	TLR10_rs4274855	0	1		0.364
3-4 <0.001 (<0.001, >999.999) 0.978	TLR1_rs5743604	1	, , ,	0.532	
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TLR10_rs11466640 0 1.00 (referent) 0.459 TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776 2 0.893 (0.55, 1.44) 0.496 3-4 <0.001 (<0.001, >999.999) 0.981 ≥1 var for one SNP 0.87 (0.59, 1.27) 0.801 ≥1 variant allele for both SNPs 0.27 (0.07, 1.03) 0.033 1 variant allele for both SNPs 0.30 (0.08, 1.11) 0.034 1 variant allele for both SNPs 0.30 (0.08, 1.11) 0.034 1 variant allele for both SNPs 0.30 (0.08, 1.11) 0.034 1 variant allele for both SNPs 0.30 (0.08, 1.11) 0.034 1 variant allele for both SNPs 0.39 (0.17, 0.89) 0.515 2 0.39 (0.17, 0.89) 0.041 0.041 2 1 variant allele for both SNPs 0.20 (0.05, 0.77) 0.025 2 1 variant allele for both SNPs 0.20 (0.05, 0.77) 0.025 2 1 var for both SNPs 0.21 (0.06, 0.80) 0.023 1RAK4_rs4251520 0 1.00 (referent) 0.831 1 TOLLIP_rs3168046 1 1.52 (0.98, 2.41) 0.982 2 1 var for one SNP 1.40 (0.90, 2.18) 0.047 </td <td></td> <td>S4 C L CND</td> <td>0.20 (0.07.4.07)</td> <td>0.004</td> <td></td>		S4 C L CND	0.20 (0.07.4.07)	0.004	
TLR1_rs5743604 1 0.81 (0.54, 1.22) 0.776 2 0.893 (0.55, 1.44) 0.496 3-4 <0.001 (<0.001, >999.999) 0.981 ≥1 var for one SNP 0.87 (0.59, 1.27) 0.801 ≥1 variant allele for both SNPs 0.27 (0.07, 1.03) 0.033 1 ≥1 var for both SNPs 0.30 (0.08, 1.11) 0.034 1 0.96 (0.66, 1.39) 0.515 0.213 1 0.96 (0.66, 1.39) 0.515 0.20 (0.01, >0.099.999) 0.978 2 0.39 (0.17, 0.89) 0.041 0.041 0.001 (<0.001, >999.999) 0.978 2 1 var for one SNP 0.91 (0.64, 1.31) 0.721 0.721 0.025 2 1 var for both SNPs 0.20 (0.05, 0.77) 0.025 0.025 2 1 var for both SNPs 0.21 (0.06, 0.80) 0.023 1RAK4_rs4251520 0 1.00 (referent) 0.831 TOLLIP_rs3168046 1 1.52 (0.98, 2.41) 0.982 2 1 var for one SNP 1.40 (0.90, 2.18) 0.047 2 1 var for one SNP 1.40 (0.90, 2.18) 0.047	TI D 10 11 1666 10		• • •	0.034	0.450
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≥1 var for one SNP		2	0.39 (0.17, 0.89)	0.041	
≥1 variant allele for both SNPs		3-4	<0.001 (<0.001, >999.999)	0.978	
≥1 var for both SNPs 0.21 (0.06, 0.80) 0.023 IRAK4_rs4251520		≥1 var for one SNP	0.91 (0.64, 1.31)	0.721	
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		≥1 var for both SNPs	0.44 (0.23, 0.86)	0.011	

SUMMARY

- 22 TLR SNP pairs were associated with PCa risk under the multiplicative and additive genetic models using Plink & SAS. However, these models lost their significance after adjusting for multiple hypothesis testing.
- The significant SNP pairs identified using Plink (P <0.0001) may require validation using advanced bioinformatics tools with the capacity to consider the impact of complex epistasis models in relation to PCa outcome predictions.

FUTURE DIRECTIONS

- Identify the joint modifying effects of TLR SNPs and lifestyle factors linked to poor gut microbiome on PCa risk using a larger and ethnically diverse sub-populations.
- Identify and evaluate the impact of additional TLR SNP pairs on PCa risk and aggressive phenotypes in the absence of main effects using Statistical Epistasis Network (SEN) and Genetic Architecture Model Emulator for Testing & Evaluating Software (GAMETES).

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