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Inflammation Polymorphisms and Prostate Cancer Risk in Jamaican Men: Role of Obesity/Body Size

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Abstract

African ancestry and obesity are associated with higher risk of prostate cancer (PC). In a pilot study, we explored interactions between obesity (as measured by waist to hip ratio (WHR)) and inflammatory SNPs in relation to PC risk among Jamaican men. This study evaluated 87 chemokine and cytokine associated SNPs in obese and normal weight cases (N=109) and controls (N=102) using a Stepwise Penalized Logistic Regression approach in multivariable analyses.

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Upon stratification by WHR (normal weight (WHR<0.90) or obese (WHR ≥ 0.90)), inheritance of CCR6 rs2023305 AG+GG (OR=1.75, p=0.007), CCR9 rs7613548 AG+GG (OR=1.71, p=0.012) and IL10ra rs2229113 AG+GG (OR=1.45, p=0.01) genotypes was associated with increase in overall or low grade (Gleason score < 7) PC risk among normal weight men. These odds were elevated among obese men who possessed the CCR5 rs1799987 AG+GG (OR=1.95, p=0.003) and RNASEL rs12135247 CT+TT genotypes (OR=1.59, p=0.05). CCR7 rs3136685 AG+GG (p=0.032) was associated with a 1.52-1.70 fold increase in the risk of high grade cancer (Gleason score ≥ 7) among obese men. CCR7 variant emerged as an important factor associated with high grade PC risk among obese men in our analyses. Overall, genetic loci found significant in normal weight men were not significant in obese men and vice-versa, partially explaining the role of obesity on PC risk among black men. Also, older age was an important risk factor both in normal weight and obese men but only with regard to low grade PC. Associations of inflammatory SNPs with obesity are suggestive and require further validation in larger cohorts to help develop an understanding of PC risk among obese and non-obese men of African descent.

Keywords

Prostate Cancer; Obesity; Waist to Hip ratio; Black; Single nucleotide polymorphisms

1. Introduction

Prostate cancer (PC) is the most common cancer as well as the leading cause of cancer-related deaths in Jamaica [Gibson, et al. 2008] where the population is primarily of African descent (92.1%). It is known that PC incidence and mortality rates are higher among black men [Greenlee, et al. 2000]. Risk is further increased in the case of obese black men as obesity plays an important role in PC progression [Vidal, et al. 2014].

Obesity is linked to low-grade chronic inflammation, a condition related to increased fatty acids, inflammatory cytokine production, and an influx of immune cells that add to the production of inflammatory mediators [Zeyda and Stulnig 2007]. Normally, white adipose tissue stores lipids and the tissue macrophages release mainly anti-inflammatory cytokines like IL-10, IL-1ra, etc [Lumeng, et al. 2008]. However, in obesity, owing to the changes in cytokine profile, the resident macrophages in the white adipose tissue release pro-inflammatory cytokines [Balkwill and Mantovani 2001]. This imbalance in inflammatory and immune surveillance pathways in chronic inflammation possibly leads to development/progression of cancer [Kidd, et al. 2012].

Among the inflammatory factors, cytokines (interleukins and TNFs) and chemokines (CC, CXC, XCL, and C-X3-C), are known to play an important role in host response to infection, inflammatory process, tumor development and metastasis [Jones, et al. 2013]. Studies suggest that single nucleotide polymorphisms (SNPs) in inflammatory related genes and altered expression play a role in development and survival of various cancers including PC [Chang, et al. 2009]. While many studies have focused on the association of PC risk with obesity, sequence variants, and other risk factors, very few reports have shed light on the underlying interactions among the aforementioned risk factors in relation to PC

susceptibility and disease progression. We have previously published on SNPs of inflammatory related genes and PC risk [Jones, et al. 2013; Kidd, et al. 2012]. Also, we have previously reported positive correlations with PC risk and Waist to Hip Ratio (WHR) in this current study population [Jackson, et al. 2010]. In this study, we further elaborate the PC and WHR relationship with respect to body size (targeting men with central/abdominal obesity by using WHR as categorizing measure) in order to uncover possible interactions among genetic variants, PC risk factors, and low/high grade PC in obese and normal weight men of African descent.

These preliminary results will shed light on the role of interactions among genetic variants and other risk factors and their joint modifying effects on PC in obese and normal weight men and would pave the way for validation in larger cohorts.

2. Methods

2.1 Subjects

The current study utilized 211 DNA samples (109 cases and 102 controls) of Jamaican black men from a larger study designed to look into the association of genetic variants and PC risk in 515 subjects (242 cases and 273 controls). These 515 individuals comprise of 211 Jamaican black men and 304 US black men [Jones, et al. 2013; Kidd, et al. 2012]. Details of the Jamaican study subset (case and control ascertainment, inclusion criteria) have been previously published elsewhere [Jackson, et al. 2012]. Briefly, patients were recruited from urology clinics at the two main tertiary hospitals and from private practitioners in the Kingston metropolitan area in Jamaica. 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

Genotyping was performed (for 43 chemokine and 44 cytokine SNPs) on 211 DNA samples (109 cases and 102 controls), as a part of collaborative project. Criteria of SNP selection, quality control, data management of genotype data, testing for Hardy Weinberg equilibrium are documented in our previously published studies [Jones, et al. 2013; Kidd, et al. 2012]. Genotyped data used in this study were combined from the above mentioned published studies for a total of 87 (43 chemokine related and 44 cytokine related) SNPs. A list of SNPs is provided in Supplementary Table 1.

2.3 Epidemiologic and Anthropometric Data

A standardized questionnaire was interview-administered. Data included demographics, medical history, dietary habits, lifestyle factors (physical activity and smoking, etc.) and sexual behavior. Physical activity was measured qualitatively using nature of work and leisure type activities. Education was categorized as primary education or less, secondary/high school education and tertiary/college according to the highest grade attended in school. Weight, hip circumference, waist circumference and height measurements were made on subjects without shoes and wearing light clothing. Weight was measured on an electronic digital scale to the nearest 0.1 kg, height with a portable stadiometer fitted with a spirit level

to 0.1 of a centimeter. Waist circumference was measured at the mid-point between the lower ribs and the iliac crest. Hip circumference was measured horizontally at the largest extension over the buttocks [Lohmann, et al. 1988]. The above mentioned measurements were used to calculate body mass index (BMI) as weight in kilograms/ square of height in meters and WHR as ratio of waist circumference to that of hips.

2.4 Statistical Analyses

The demographic characteristics and differences between cases and controls in family/ medical history, age, BMI, WHR, smoking status, education and marital status were examined for the study population. Chi square statistics or Fishers exact test were used for categorical variables and the Wilcoxon Rank-Sum Test was used for continuous variables using the STATA SE (13.0), StataCorp, College Station, TX [StataCorp 2013]. WHR was calculated and categorized as normal weight (WHR<0.90) or obese (WHR ≥ 0.90) [WHO, 2011]. Cases were stratified into low grade (Gleason score of <7) and high grade (Gleason score of ≥ 7) PC.

We calculated statistical power for our study to detect significant association between inflammatory related SNPs and PC risk using Power for Genetic Association v.2 software [Menashe, et al. 2008]. The parameters used were: minor allele frequency 23%, National Cancer Institute's estimate of PC prevalence based on African-American US men (0.74%), false positive rate of 5%, number of cases (n =109). Effective degree of freedom was calculated for our genotype data using the same software which was found to be 62. Based on our sample size of 109 cases and 102 controls, we had 80 % power to detect genetic associations with odds ratio ≥ 2. The association between PC risk and inflammatory SNPs, expressed as odds ratios (ORs) and corresponding 95% confidence intervals (CIs), were estimated using logistic regression models in STATA SE (13.0), adjusting for age. The analyses were run separately after stratifying for WHR. Logistic Regression (LR) analyses for genetic variants and PC risk were conducted using major/common genotype as the reference category.

The SNPs from the age adjusted LR analysis found statistically significant in association with PC risk were used for further analyses. Pair-wise linkage disequilibrium (LD) was calculated using STATA to check any random association between alleles. Pair-wise LD was measured in terms of r^2 and D' values. Among the SNP pairs with high LD scores ($D' > 0.90$), only one SNP was retained for further analyses. Inflammatory markers IL6, CRP and TNF were examined for their relationship with PC using univariate logistic regression. None of the markers were found significant and were not included for further analyses.

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 StepAIC package in R [Park and Hastie 2008; Team 2008]. Penalized LR stabilizes the coefficients in a LR model by adding a penalty term to the likelihood function which favors shrunken coefficients. This method allows for graceful handling of multicollinearity and sparse cells in multifactor contingency tables, which are typical problems encountered by regular LR when considering high-order interactions. Regularization parameter λ [Park and Hastie 2008] was chosen by running Cross Validation

(CV) analyses with different values of λ using the StepPlr package. Best λ corresponding to the lowest value of Akaike Information Criteria (AIC) as calculated by CV was selected. In all multivariable models the following variables were included in the analyses: age (as continuous variable), smoking status (yes/no), and physical activity (inactive/active). The Stepwise penalized logistic regression (StepPlr) was performed for the total population as well as after stratifying according to WHR and cancer grade (low/high).

3. Results

The characteristics for the study population (109 cases and 102 controls) are summarized in Table 1. PC cases were older ($p < 0.0001$) and had significantly higher PSA levels ($p < 0.0009$). Controls were less physically active than cases ($p = 0.005$). Cases were more likely to be smokers and controls were more likely to be reported as non-smokers ($p < 0.0001$). Cases and controls did not differ with respect to BMI ($p = 0.6723$), WHR ($p = 0.06$), family history of PC ($p = 0.516$), education ($p = 0.094$) and marital status ($p = 0.08$).

3.1 Single gene effects

Our genetic analyses were based on 211 genotyped samples (109 cases and 102 controls). In the primary analyses using logistic regression, ten of the 87 chemokine and inflammatory SNPs were found to be associated with PC risk (Table 2). The LR analyses for age adjusted models, stratified by WHR (< 0.90 or ≥ 0.90) indicated that for participants with WHR < 0.90 , a greater than two to four-fold increased PC risk was associated with possession of variant chemokine and interleukin receptor alleles: CCR9 (rs7613548 AG+GG, rs2286486 AG+GG), CCR6 (rs2023305 AG+GG, rs1556413 CT+TT) and IL10RA (rs2229113 AG+GG); however, a reduced PC risk was associated with the interleukin receptor IL1r1 (rs219275 AC+CC) sequence variant. Among participants with WHR ≥ 0.90 , greater than three to four-fold increased PC risk was associated with variants CCR5 (rs1799987 AG+GG, rs1799988 AG+GG), CCR7 (rs3136685 AG+GG) and RNASEL (rs12135247 CT+TT). Pair wise linkage disequilibrium analysis for the above 10 SNP loci indicated strong linkage for three SNP pairs CCR5 rs1799988/CCR5 rs1799987, CCR6 rs2023305/CCR6 rs1556413, and CCR9 rs7613548/CCR9 rs2286486 with LD scores > 0.90 , as depicted in Supplementary Table 2.

In order to avoid the issue of collinearity in multivariable models, one SNP each from the 3 pairs was omitted (CCR5 rs1799988, CCR6 rs1556413 and CCR9 rs2286486) from the penalized LR modeling to ensure accurate estimates of regression coefficients. The remaining seven SNP loci were analyzed to test gene-environment interactions using the StepPlr method. The cross-validation analyses guided us in selecting an appropriate value of λ for our data which was found to be 1.

3.2 Multivariable Analyses

The results of multivariable models with age, smoking status, physical activity and SNPs analyzed using stepwise penalized logistic regression approach for total population as well as stratified population for WHR and cancer grade are summarized in Table 3 and Table 4.

This method yielded results in terms of main effects and interactive effects of tested SNP loci and other factors in association with PC risk.

(a) WHR<0.90—Among individuals who had a WHR < 0.90 (Table 3), main effects associated with total PC risk included, age (OR=1.15, 95% CI: 1.09-1.21, p-value= <0.001) along with genetic loci variants inheritance of CCR6 rs2023305 AG+GG (OR= 1.75, 95% CI: 1.17-2.64, p-value=0.007), CCR9 rs7613548 AG+GG (OR=1.71, 95% CI: 1.13-2.60, p-value= 0.012), and IL10RA rs2229113 AG+GG (OR= 1.45, 95% CI: 1.09-1.95, p-value= 0.01) genotypes. An interaction between no physical activity and IL10RA rs2229113 AA genotype was associated with lower PC risk (OR= 0.48, 95% CI: 0.31-0.77, p-value< 0.001).

With stratification by cancer grade, low grade PC was found to be associated with age (OR=1.06, 95% CI: 1.03-1.10, p-value=0.001) and inheritance of the IL10RA rs2229113 AG+GG genotype (OR=1.38, 95% CI: 0.99-1.94, p-value=0.058). Age was also observed to have an interactive effect with CCR9 rs7613548 AG+GG (OR=1.05, 95% CI: 1.03-1.08, p-value 0.001) and also with CCR6 rs2023305 AG+GG (OR=1.05, 95% CI: 1.02-1.07, p-value 0.001) on the risk of low grade PC. Similar to total PC, an interaction between no physical activity and IL10RA rs2229113 (AA) genotype was identified again in relation to decrease in low grade PC risk (OR= 0.51, 95% CI: 0.31-0.85, p-value= 0.010). Stratified analyses for high grade PC for people with WHR<0.90, did not result in any statistically significant interaction among physical activity, age and genotypes.

(b) WHR ≥ 0.90—Among individuals with WHR ≥ 0.90 (Table 4), total PC risk in these obese men was influenced by possession of CCR5 rs1799987 AG+GG (OR= 1.95, 95% CI: 1.26-3.01, p-value=0.003) and CCR7 rs3136685 AG+GG (OR= 1.70, 95% CI: 1.06-2.73, p-value=0.028) genotypes. An interactive effect of physical inactivity and wild-type genotype of loci IL1R1 rs2192752 AA (p-value=0.008) was significantly associated with decreased risk of total PC in obese men.

After stratifying the population for cancer grade, age (OR= 1.08, 95% CI: 1.01-1.16, p-value= 0.018), RNASEL rs12135247 (CT+TT) (OR= 1.59, 95% CI: 0.99-2.56, p-value=0.054) and CCR5 rs1799987 AG+GG (OR= 1.70, 95% CI: 1.05-2.76, p-value= 0.03) were the main effects linked with increased risk of low grade PC. In addition, an interaction between age and physical inactivity (OR= 1.04, p-value=0.033) and interaction among smoking, physical activeness and age (OR= 1.07, p-value=0.016) were seen to be associated with increased risk of low grade PC. In the case of high grade PC, CCR7 rs3136685 variant genotype AG+GG was significantly related with increased PC risk (OR= 1.52, 95% CI: 1.03-2.23, p-value=0.032). An interaction between chemokine receptor genotypes (CCR6 rs2023305 AA and CCR7 rs3136685 AG+GG) (p-value=0.019) as well as inheritance of the CCR5 rs1799987 AG+GG genotype and age (p-value=0.026) seem to play a role in increased risk for high grade PC.

4. Discussion

In this pilot study we aimed to explore the behavior and interactions of SNP loci and other risk factors with respect to PC risk in a case control study among Jamaican men stratified

according to body size using WHR. Much research denotes that the WHR is the superior health risk-categorizing indicator, as it is a measure of central adiposity which predicts risk for diseases like cardiovascular, diabetes and PC better than BMI [Jackson, et al. 2010; Srikanthan, et al. 2009]. Further, diseases are largely affected by gene-gene or gene-environment interactions, rather than by a single gene/factor. Hence, it becomes imperative to explore any underlying interaction structures among the risk factors that concern disease susceptibility. Logistic regression is the most commonly used tool to find the association between risk factors and disease risk. However, in case of SNP data with 2-3 levels of genotype factors along with other confounding variables, problems of overfitting models may arise especially if data size is small [Park and Hastie 2008]. The 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 gene-gene interaction models. StepPlr analyses resulted in numerous gene-gene and gene-environment interactions; however, the discussion will be limited to only statistically significant results.

Among normal weight people, the variants associated with total PC were CCR6 rs2023305 AG+GG, CCR9 rs7613548 AG+GG and IL10RA rs2229113 AG+GG. IL10RA rs2229113 AG+GG was also associated with increased risk of low grade PC. Chemokines and their corresponding receptors play a role in leukocyte trafficking and host defense; however, tumor cells acquire this property and exploit the chemokine and their receptors for their survival and dissemination [Wang, et al. 1998]. Reports established CCR9 is highly expressed by PC cell lines and affect cancer cell migration, invasion, and MMP expression, which together may affect PC metastasis [Singh, et al. 2004]. Similarly, the levels of CCR6 were upregulated in colorectal cancer cell lines and play a critical role in metastasis [Liu, et al. 2014]. Variations in IL10RA, the gene encoding receptor for anti-inflammatory cytokine interleukin-10, have been associated with cancer risk in some studies [Nieters, et al. 2006]. Although these genes and their sequence variants have been associated with cancer, our study reports that upon stratification of population by body size, the significance of these gene variants were limited to normal weight men.

Older age plays an important role in elevating the risk for total and low grade PC; however, the effect was not significant in case of high grade cancer. A higher incidence of microscopic foci of prostate cancer is found among men with increasing age. In low grade cancer cases, the cancer tends to grow very slowly and is diagnosed usually at older ages. However, a number of younger men are diagnosed with aggressive/ high grade PC which is not so age dependent and is often linked with genetic mutations [Salinas, et al. 2014]. Results from our analyses also support this theory, since the risk of high grade PC in both obese and non obese subjects was age independent but was modulated upon possession of genetic variants from genes CCR5 and CCR7 in obese subjects. As we know, African ancestry is a recognized risk factor for PC, younger Jamaican males possessing CCR5 and CCR7 polymorphisms would be more vulnerable to the aggressive form of the disease especially if they happen to be obese by WHR measure. These results suggest that this group may benefit to a greater extent than others from evaluation of genetic risk in terms of PC.

In addition to the single main effects resulting from genetic variants, a statistically significant interaction was observed between age and sequence variants of CCR6 and CCR9

which led to a slight increase in the risk of low grade PC. These findings were not surprising, since age, CCR6, and CCR9 variants were found associated with increase in PC risk. Another important interaction was seen between physical inactivity and IL10RA rs2229113 AA genotype, resulting in decreased risk of total and low grade PC. Physical activity is recommended as primary prevention in PC; however, a couple of studies demonstrated an inverse relationship between the level of physical activity and PC risk [Friedenreich and Thune 2001; Oliveria and Lee 1997]. Physical activity can have different influences on carcinogenesis, depending on energy supply and the age of the subject as well as strength, frequency, and length of exercise [Na and Oliynyk 2011]. Our observation of alterations in PC susceptibility among senior adults (mean ages 60 and 70 years old among controls and cases, respectively) who possessed the IL10RA rs2229113 locus in a physically inactive environment may be related to *IL10RA*'s role in mediating the immunosuppressive signal of interleukin 10, followed by inhibition of pro-inflammatory cytokine synthesis, and ultimately a reduction in PC. 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 (ROS) which can lead to DNA damage, or may support tumorigenesis by triggering pro-inflammatory signaling [Na and Oliynyk 2011]. Also, evidences indicate increasing levels of testosterone with exercise which have also been associated with increased risk of PC [Zmuda, et al. 1996].

Investigation of the obese population revealed an increase in the risk of developing PC among carriers of the CCR5 rs1799987 AG+GG and CCR7 rs3136685 AG+GG genotypes. Upon stratification by cancer grade, CCR5 rs1799987 AG+GG and RNASEL rs12135247CT+TT genotypes were associated with low grade cancer and CCR7 rs3136685 AG+GG was associated with high grade PC risk. Reports indicate that over expression of CCR5 has been detected in PC tissue and polymorphisms of this gene are known to be associated with risk of PC in Jamaican men [DZ 2014; Konig, et al. 2004]. We found that CCR5 rs1799987 AG+GG variant genotype was associated with a 1.70-1.90 fold increase in risk of total and low grade PC among obese men. RNASEL rs12135247 variant genotype CT+TT was associated with 1.59 fold increase in low grade PC risk among obese men. Mutations in RNASEL have been documented to be linked with head and neck, breast, and cervical cancers and sequence variants of RNASEL have also been known to be associated with PC risk in men of European descent [Casey, et al. 2002; Madsen, et al. 2008].

CCR7 gene expression mediates the metastatic behavior of colorectal cancer and prognosis in patients with breast cancer, osteosarcoma and lymphatic metastasis [Takanami 2003]. In addition, high expression of CCR7 in tumors of African-American PC patients when compared with European-American patients has been reported [Wallace, et al. 2008]. *In vivo* study correlated increased level of CCR7 gene expression in intra-abdominal adipose tissue of obese mice [Lee, et al. 2009]. Locus CCR7 rs3136685 has been found to be significantly associated with PC risk among men of African descent [DZ 2014]. Our results suggest a link between CCR7 variant and aggressive PC (1.52 fold increase) in obese black men. Further we show that inheritance of CCR7 rs3136685 variant along with CCR6 rs2023305 AA genotype results in interplay of genetic factors ultimately affecting the risk of high grade PC by 1.81 fold. The aforementioned loci have previously been linked to prostate cancer and

other cancers, whereas, our study specifically links these loci with PC risk among men falling in obese group by WHR measure.

In addition, interactive effects of age and physical inactivity minimally increased the risk of low grade PC by 1.04 fold. In this case, lack of physical activity in obese men in addition to their increasing age made them susceptible to the risk of low grade PC. However, in another significant interaction, among older men who were physically active and current smokers, there was a minor increase in risk of low grade PC by 1.07 fold. Smoking is a known risk factor for PC and cigarette smoke is known to contain ROS which cause an enhancement in oxidative stress [Aoshiba and Nagai 2003]. Also, as previously indicated, exhaustive physical activity is related to accumulation of ROS, a combination of smoking and heavy physical activity among older men may render a person susceptible to the risk of low grade PC. Our age adjusted analyses showed that IL1R1 rs2192752 was associated with an 84% decrease in the risk of PC among normal weight men (WHR <0.90). Also, among obese men (WHR ≥ 0.90), low/no physical activity in interplay with inheritance of the AA genotype at IL1R1 rs2192752 locus, which is a receptor for pro-inflammatory cytokine, shows a protective effect (decreased risk by 0.54 fold) against the risk of total PC, since, depending on the intensity, duration and frequency of physical activity, immune functions can be suppressed or enhanced [Norman, et al. 2002].

Strengths of our study included a population with PC cases with measured body size using WHR to focus on men with abdominal adiposity. In addition our samples had nearly equal representation of high/low grade PC cases and of obese/non obese subjects. The analysis used PLR which is known to uncover underlying interactions among risk factors compared to the commonly used LR models, particularly when the sample size is small. Results from this study suggest that interactions of polymorphic inflammatory genes with different risk factors are affected by obese and non-obese environments. Results of this pilot study warrant further validation in larger studies. Overall, our study reports association of CCR5 rs1799987 and CCR7 rs3136685 loci with PC risk among Jamaican men, specifically with high grade PC. Our findings tentatively point towards the importance of the development of genetic biomarkers for the identification of men at greater risk of developing an aggressive form of PC. Our results have not been tested using significance thresholds for multiple testing, since adjustments in the significance level may not always be warranted, especially when the purpose of the study involves screening or hypothesis generation [Roback 2005]. The results from this study are suggestive and further testing of the significant interactions reported here would be carried out on a larger scale using a study population of African American men with obesity and genetic data.

5. Conclusion

We noted that genetic loci found significant in normal weight men were not significant in obese men and vice-versa, partially explaining the role of obesity on PC risk among black men. Age was an important risk factor both in normal weight and obese men but only with regard to low grade PC. Physical inactivity interaction with age or genetic variants decreased the risk of low grade PC. Unlike low grade PC where the risk was primarily associated with interactions among age, smoking status, physical activity and genetic variants, high grade

PC was mainly linked with genetic interactions within the CCR6-CCR7 axis. The CCR7 variant emerged as an important factor associated with high grade PC risk among men with abdominal obesity. A major objective of evaluating biomarkers for the detection of PC is to distinguish patients who will eventually develop metastases (high grade cancer) from those with more indolent forms. The joint analysis of variants of inflammatory related genes with biological risk factors is expected to develop some understanding of the interplay between various risk factors involved. The analyses are also expected to help in prediction of PC risk in obese and non-obese men of African descent in larger size studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PC	Prostate cancer
WHR	Waist to hip ratio
BMI	Body Mass index
OR	Odds ratio
CI	Confidence interval
LR	Logistic regression
StepP_{lr}	Stepwise penalized logistic regression
CV	Cross validation
AIC	Akaike Information criterion

Highlights

- 7 SNPs on inflammatory related genes were found associated in Prostate cancer cases and controls.
- Stratification by body size: genetic loci significant in normal weight men were not significant in obese men and vice-versa.
- Gene x environment interaction analyses suggest age is related to low grade PC and genetic variants play important role in high grade disease.
- CCR7 variant was associated with high grade PC risk among obese men.

Table 1

Characteristics of study population by case/control status.

Characteristics	Cases	Controls	p value
Number of Participants	109	102	-
Age (years), Median (range)	70 (49-80)	60 (40-80)	<0.0001
Waist Hip Ratio, Mean (S.D)	0.90 (0.07)	0.88 (0.09)	0.06
BMI (kg/m2), Mean (S.D)	25.50 (4.60)	25.23 (4.05)	0.6723
PSA (ng/ml), Median (range)	35.3 (4.0-10000)	1.20 (0.20-4.0)	<0.0009
Smoking Status, n (%)			<0.0001
No	25 (22.9)	47(46.07)	
Yes	83 (76.14)	55 (53.92)	
Family History of PC, n (%)			0.516
No	92(84.4)	90(88.23)	
Yes	16(14.67)	12(11.76)	
Physical Activity, n (%)			0.005
Inactive	23 (21.1)	22 (21.5)	
Moderately Inactive	17 (15.59)	32 (31.37)	
Moderately Active	44 (40.36)	39 (38.23)	
Active	24 (22.01)	8 (7.84)	
Education, n (%)			0.094
Primary or less	96 (88.07)	79 (77.45)	
Secondary	7 (6.42)	15 (14.70)	
Tertiary	4 (3.66)	6 (5.88)	
Marital Status, n (%)			0.08
Married	61 (55.96)	65 (63.72)	
Previously Married	20 (18.34)	8 (7.84)	
Others	27 (24.7)	28 (27.45)	
Gleason Score, n (%)			
6	45 (41.67)		
7	37 (34.26)		
8	13 (12.04)		
9	10 (9.26)		
10	2 (1.85)		
missing	2 (1.85)		

Table 2
Sequence variants with significant P-values and association (age adjusted) with Prostate cancer risk in study population stratified by waist to hip ratio

Genes	dbSNP ID	WHR < 0.90		WHR 0.90	
		OR (95% CI)	P value	OR (95% CI)	P value
CCR9	rs7613548	4.36 (1.47-12.91)	0.008	-	-
CCR6	rs2023305	3.54 (1.31-9.57)	0.013	-	-
IL10ra	rs2229113	3.14 (1.80-8.21)	0.019	-	-
CCR9	rs2286486	2.48 (1.04-5.95)	0.04	-	-
CCR6	rs1556413	2.61 (1.03-6.60)	0.042	-	-
IL1rl	rs2192752	0.16 (0.02-0.93)	0.042	-	-
CCR5	rs1799987	-	-	3.99 (1.35-11.76)	0.012
CCR5	rs1799988	-	-	3.99 (1.35-11.76)	0.012
RNASEL	rs12135247	-	-	3.29 (1.15-9.40)	0.026
CCR7	rs3136685	-	-	4.61 (1.11-19.12)	0.035

Table 3
Association and Interactions between chemokine, cytokine related SNPs/environmental factors and prostate cancer risk among men with WHR<0.90

Prostate Cancer (n)	WHR < 0.90							
	Main effects/Genes	dbSNP ID	Location	Genotype	Interactions	OR	95% CI	P val
Total PC (n = 62)	Age					1.15	1.09-1.21	<0.001
	CCR6	rs2023305	5' near gene	AA		0.57	0.38-0.86	0.01
				AG+GG		1.75	1.17-2.64	0.01
	CCR9	rs7613548	Intron 2	AA		0.58	0.38-0.89	0.01
				AG+GG		1.71	1.13-2.60	0.01
	IL10ra	rs2229113	Exon 7	AA		0.68	0.51-0.91	0.01
				AG+GG		1.45	1.09-1.95	0.01
					Inactive:IL10ra_rs2229113(AA)	0.48	0.31-0.77	<0.001
					Inactive:IL10ra_rs2229113(AG+GG)	1.45	0.86-2.43	0.16
					Active:IL10ra_rs2229113(AA)	1.41	0.92-2.16	0.12
					Active:IL10ra_rs2229113(AG+GG)	1.01	0.62-1.65	0.97
Low Grade PC (n = 25)	Age					1.06	1.03-1.10	<0.001
	IL10ra	rs2229113	Exon 7	AA		0.72	0.52-1.01	0.06
				AG+GG		1.38	0.99-1.94	0.06
					CCR9_rs7613548(AG/GG):Age	1.05	1.03-1.08	<0.001
					CCR6_rs2023305(AG/GG):Age	1.05	1.02-1.07	<0.001
					CCR6_rs2023305(AA):Age	1.02	0.99-1.03	0.07
					Inactive:IL10ra_rs2229113(AA)	0.51	0.31-0.85	0.01
					Inactive:IL10ra_rs2229113(AG+GG)	1.36	0.86-2.14	0.18
					Active:IL10ra_rs2229113(AA)	1.40	0.83-2.35	0.20
					Active:IL10ra_rs2229113(AG+GG)	1.02	0.65-1.60	0.94
					CCR9_rs7613548(AA):Age	1.01	0.98-1.03	0.39
High Grade PC (n = 37)	Age					1.04	0.95-1.15	0.36
					Inactive:Age	0.96	0.83-1.12	0.62

Prostate Cancer (n)	WHR < 0.90						
	Main effects/Genes	dbSNP ID	Location	Genotype	Interactions	OR	P val
					Active:Age	1.08	0.16
					IL1rl_rs2192752(AA):Inactive:Age	1.14	0.36
					IL1rl_rs2192752(AA):Active:Age	1.00	0.94
					IL1rl_rs2192752(AC+CC):Inactive:Age	0.85	0.39
					IL1rl_rs2192752(AC+CC):Active:Age	1.08	0.41
					CCR6_rs2023305(AA):Inactive:Age	1.03	0.70
					CCR6_rs2023305(AA):Active:Age	1.03	0.65
					CCR6_rs2023305(AG+GG):Inactive:Age	0.94	0.57
					CCR6_rs2023305(AG+GG):Active:Age	1.06	0.57
					CCR5_rs1799987(AA):CCR6_rs2023305(AA):Inactive:Age	1.05	0.25
					CCR5_rs1799987(AA):CCR6_rs2023305(AA):Active:Age	1.00	0.94
					CCR5_rs1799987(AA):CCR6_rs2023305(AG+GG):Inactive:Age	0.95	0.67
					CCR5_rs1799987(AA):CCR6_rs2023305(AG+GG):Active:Age	0.94	0.67
					CCR5_rs1799987(AG+GG):CCR6_rs2023305(AA):Inactive:Age	0.98	0.63
					CCR5_rs1799987(AG+GG):CCR6_rs2023305(AA):Active:Age	1.03	0.35
					CCR5_rs1799987(AG+GG):CCR6_rs2023305(AA):Inactive:Age	0.99	0.92
					CCR5_rs1799987(AG+GG):CCR6_rs2023305(AG+GG):Active:Age	1.13	0.36

* Number of cases = 62 and controls = 60; Inactive/active refers to physical inactivity/activity.

Table 4
Association and Interactions between chemokine, cytokine related SNPs/environmental factors and prostate cancer risk among men with WHR 0.90

Prostate Cancer (n)	WHR 0.90						
	Main effects/Genes	dbSNP ID	Location	Genotype	Interactions	OR	P val
Total PC (n = 44)	Age					1.10	0.011
	CCR5	rs1799987	5' UTR	AA		0.51	0.003
				AG+GG		1.95	0.003
	CCR7	rs3136685	Intron 1	AA		0.59	0.028
				AG+GG		1.70	0.028
	Inactive					0.82	0.206
	Active					1.22	0.206
					IL1 rs2192752(AA):Inactive	0.54	0.008
					IL1 rs2192752(AA): Active	1.49	0.087
					IL1 rs2192752(AC+CC):Inactive	1.52	0.095
Low Grade PC (n = 18)					IL 1 rs2192752(AC+CC) / Active	0.82	0.43
	Age					1.08	0.018
	RNASEL	rs12135247	UTR 3'	CC		0.63	0.054
				CT+TT		1.59	0.054
	CCR5	rs1799987	5' UTR	AA		0.59	0.03
				AG+GG		1.70	0.03
					Inactive:Age	1.04	0.033
					Smoke:Active:Age	1.07	0.016
					Active:Age	1.04	0.07
					NoSmoke:Inactive:Age	1.03	0.062
High Grade PC (n = 26)					NoSmoke:Active:Age	0.98	0.453
					Smoke:Inactive:Age	1.01	0.55
	CCR7	rs3136685	Intron 1	AA		0.66	0.032
				AG+GG		1.52	0.032
	CCR5	rs1799987		AA		0.94	0.373

Prostate Cancer (n)	WHR 0.90						
	Main effects/Genes	dbSNP ID	Location	Genotype	Interactions	OR	95% CI
				AG+GG		1.07	0.92-1.23
	Age					1.04	0.98-1.09
					CCR6rs2023305(AA);CCR7rs3136685(AG+GG)	1.81	1.10-2.97
					Age: CCR5_rs1799987(AG+GG)	1.03	1.00-1.06
					Age:CCR5_rs1 799987(AA)	1.00	0.97-1.03
					CCR6_rs2023305(AA);CCR7_rs3136685(AA)	0.70	0.44-1.10
					CCR6_rs2023305(AG+GG);CCR7_rs3136685(AA)	0.94	0.58-1.49
					CCR6_rs2023305(AG+GG);CCR7_rs3136685(AG+GG)	0.84	0.50-1.40

* Number of cases = 44 and controls = 37; Inactive/active refers to physical inactivity/activity.