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ORIGINAL PAPER

Polymorphisms in oxidative stress pathway genes and prostate cancer risk

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Abstract

Purpose Age-related factors including oxidative stress play an important role in prostate carcinogenesis. We hypothesize that germline single-nucleotide polymorphisms (SNPs) in oxidative stress pathway are associated with prostate cancer (PCa) risk. In this study, we aim to examine which of these SNPs is associated with PCa.

Methods Participants included in this analyses came from the "Genetic Susceptibility, Environment and Prostate Cancer Risk Study" conducted at the Veterans Affairs Portland Health Care System. After applying exclusion criteria, 231 PCa cases and 382 prostate biopsy-negative controls who had genotyping data on twenty-two single-nucleotide polymorphisms (SNPs) in six genes (*MAPK14, NRF2, CAT, GPX1, GSTP1, SOD2,* and *XDH*) associated with oxidative stress pathway were included in the analyses. The genotyping of SNPs was conducted by the Illumina BeadXpress VeraCode platform. We investigated these SNPs in relation to overall and aggressive PCa risk using logistic regression models controlling for relevant covariates.

Results One SNP in the *MAPK14* (rs851023) was significantly associated with incident PCa risk. Compared to men carrying two copies of allele A, the presence of one or two copies of the G allele was associated with decreased risk of PCa [OR (95% CI) 0.19 (0.06–0.51)]. There was no statistically significant association between other SNPs in the *NRF2, CAT, GPX1, GSTP1, SOD2*, and *XDH* genes and PCa risk.

Conclusions The *MAPK14* gene SNP rs851023 was associated with PCa and aggressive PCa risk after multiple comparison adjustment. Further studies in other populations or functional studies are needed to validate the finding.

Keywords Prostate cancer · SNP · Oxidative stress genes

Abbreviations

ANOVA	Analysis of variance
BIC	Bayesian information criterion
BMI	Body mass index

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95% CI	95% confidence interval
GSEP	Genetic Susceptibility, Environment and Prostate Cancer Risk
NCI	National Cancer Institute
OHSU	Oregon Health & Science University
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate-specific antigen
PSAD	Prostate-specific antigen density
SE	Standard Error
VAPHCS	VA Portland Health Care System

Introduction

The risk of developing prostate cancer increases dramatically with age [1]. Oxidative stress contributes to the aging process and affects all aspects of cellular functions that are associated with prostate cancer [2]. The importance of oxidative stress in prostate cancer carcinogenesis has been suggested in many case–control studies. A systematic review including 23 case–control studies with a total of 6,439 participants showed that prostate cancer patients had significantly higher level of oxidative stress markers (mostly at protein level) [3].

Oxidative stress, resulting from an imbalance between pro-oxidants and antioxidants, has been recognized as a consequence of aging directly related to lifespan [4]. Evidence from both in vitro and in vivo studies has shown oxidative stress as a major pathway involved in prostate cancer and other prostate diseases [5]. Free radicals [including reactive nitrogen species and reactive oxygen species (ROS)] are formed by normal metabolism and as a consequence of endogenous exposure to xenobiotics and other carcinogens. Transient increases in ROS play an important regulatory function, but sustained high levels of ROS accumulate and attack all cellular macromolecules, including proteins, DNA, and lipids, resulting in an increased cellular pro-oxidant–antioxidant imbalance and accumulation of multiple forms of DNA damage, including DNA adducts and enzyme modifications.

Mammalian cells possess a complex defense system to protect against oxidative stress induced damage. Corresponding to increased oxidative stress, decreased antioxidant enzyme activities or decreased anti-oxidative stress activities have been shown in prostate cancer [6]. Many key genes and proteins have been involved in this system. A first and crucial step in this oxidative stress response system is the activation of the transcription factor NF-E2 related factor-2 (Nrf2). Nrf2 is activated by numerous dietary phytochemicals including curcumin, catechins, resveratrol, and isothiocyanates in the presence of oxidative stimuli. Following activation, Nrf2 is released from its cytosolic repressor Kelch-like ECH-associated protein 1 (KEAP1) and translocates to the nucleus where it binds to the antioxidant response elements (AREs) found in the promoter regions of genes responsible for encoding detoxifying and antioxidant enzymes. Thus, the binding of Nrf2 to ARE results in the activation of the cellular antioxidant defense, which consists of intrinsic and extrinsic antioxidant enzymes and phase II detoxifying enzymes. Antioxidant enzymes, including superoxide dismutase (SOD), glutathione (GPX), and catalase (CAT), function to either directly quench (deactivate) ROS or stimulate the phase II detoxifying enzymes resulting in removal of ROS. Phase II detoxifying enzymes (GSTs, UDPGs, and others) metabolize carcinogens (xenobiotics) or ROS intermediates to allow for their excretion. Mitogen-activated protein kinase (MAPK)14 pathway is involved in modulation of glucose metabolism that limits ROS production and autophagy activation [7]. Xanthine dehydrogenase (XDH) is a rate-limiting enzyme in oxidative purine metabolism and activation of XDH generates oxidative stress [8].

In this study, we examined several polymorphic variants in genes involved in the oxidative stress response pathway (*MAPK14, NRF2, CAT, GPX1, GSTP1, SOD2*, and *XDH*) that might be associated with an increase in prostate cancer susceptibility from data collected in an epidemiological study—the Genetic Susceptibility, Environment, and Prostate Cancer Risk (GSEP) study. Results from the analyses will provide evidence about biological mechanisms for prostate cancer etiology.

Methods

Participants

Subjects in the GSEP study were recruited from the urology clinic at the Veterans Affairs Portland Health Care System (VAPHCS). The primary aim of the GSEP study was to determine the independent and joint effects of dietary exposures and genetic variables on prostate cancer risk. From July 2008 to December 2012, 1839 potential eligible subjects were screened, with 748 completing informed consent and interviews. One hundred and thirty-five men were excluded from the analysis due to missing data on prostate biopsy results ($n=18$), no biopsy ($n=40$), prostatic intraepithelial neoplasia (PIN) diagnosis ($n=53$), and missing data on genotyping results ($n=24$). A total of 613 subjects were included in the current analysis with 231 of them prostate cancer patients and 382 prostate biopsy-negative controls. The study sample size flowchart is depicted in Fig. 1.

Study design and data collection

Men referred for a prostate biopsy to the VAPHCS urology clinic were recruited by the study urologist and staff members prior to undergoing biopsy. Upon successful telephone contact, participants could join the study in person or remotely. For those consented in-person, the study staff

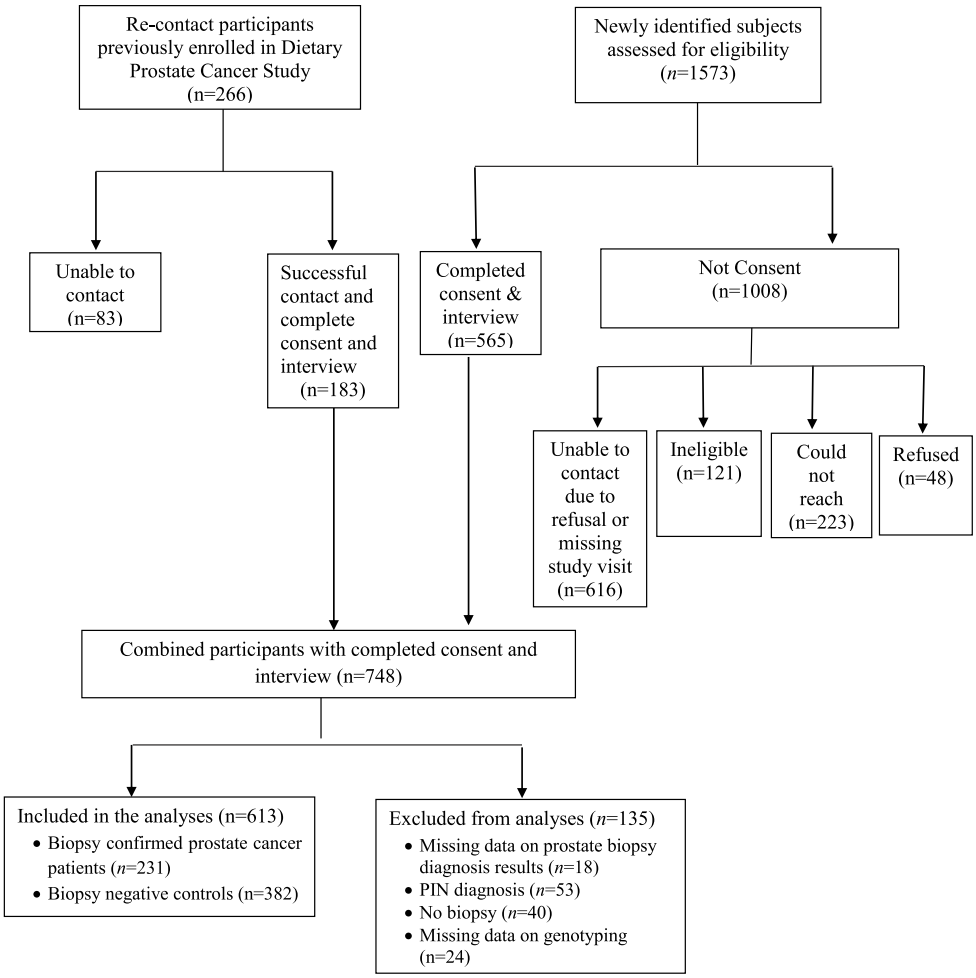

Fig. 1 Study flow chart

asked them to complete a "Changes in Diet, Prescriptions, Supplemental and Herbal Remedies" questionnaire, the "Diet History Questionnaire (DHQ)" and the Case Western University's (CWU) Genetic Risk Easy Assessment Tool (GREAT) Family History questionnaire via a secure website, post or in person, depending on participant preference. Men not able to come to the VAPHCS for consent were able to join the study remotely. Study coordinators would (1) send a blank consent form to interested men, (2) schedule a 30-min phone call to review the consent form with the subject (with a witness nearby), and (3) use a VA-approved, secure method (UPS) to send informed consent form with a pre-paid return envelope and collect the signed, witnessed consent form. Once study staff received the signed consent form, s/he mailed study materials to the participant, including (1) instructions for completing the study tasks, (2) an individualized login to a secure HIPAA-compliant website, where subjects could access the GREAT Family History questionnaire (must be completed online) and DHQ, (3) a paper copy of the DHQ, (4) an addressed return envelope (only if they prefer a hardcopy DHQ over the online questionnaire), and (5) the saliva self-collection kit with instructions and a pre-paid and pre-addressed return envelope.

Biological specimen collections

Salivary samples were collected from all participants using Oragene™ DNA Self-Collection Kit at the time of consent. These kits were returned to the Clinical and Translational Research Center (CTRC) Core Laboratory for processing, DNA extraction, analyses, and storage. Our saliva collection rate was 97% (27/744). However, we could not obtain enough saliva from some patients taking certain medications that would dry out the mouth or lived long distance away. The saliva sample is stable in room temperature and the amount of DNA extracted from a 2-mL saliva sample generated a median amount of 100 µg DNA. Participants who (1) moved away from the VAPHCS area, (2) had no plans to return to the VAPHCS for any reason, and (3) agreed to participate in the GSEP study only provided saliva samples if they participate long distance.

Saliva DNA purification procedure

The saliva sample in the Oragene™ vial was incubated at 50 °C in a water bath for 1 h and then divided into four 1.5-mL microcentrifuge tubes, each containing approximately 1 mL of sample. 40 µL (1/25th volume) of Oragene™ purifier was added to each tube and mixed gently by inversion and incubated on ice for 10 min. Samples were then centrifuged for 3 min at 15,000×*g* (13,000 rpm) at room temperature and supernatants were combined into one 15-mL centrifuge tube. An equal volume of 95% ethanol was added to the supernatant and mixed gently by inversion 5 times. Following incubation for 10 min at room temperature, the tube was centrifuged 10 min at 1,100×*g* (3,500 rpm) to pellet the precipitated DNA and the supernatant was discarded. The DNA pellet was then resuspended in 500 µL of TE and incubated at 50 °C for 10 min to fully dissolve the DNA. The DNA was then quantitated by UV spectrophotometry and stored at − 80 °C.

Selection of genes and SNPs for genotyping

Prior to genotyping, we re-applied the following criteria for selection of genes of highest priority, such that our list includes genes with (1) amino acid substitution variants, especially variants predicted to impact protein structure/activity, (2) variants in regulatory sites, (3) extent of sequence variation, and (4) centrality to the antioxidant response pathway or the cytokine/cytokine receptor axis. The availability of data from other studies was also included in the selection process. This gene selection strategy maximized the informativeness and utility of those genes selected for our question of interest. We focused our evaluation on the seven genes in Table 1, whose role in the oxidative stress response is supported by the literature, and all the selected SNPs had call rates over 95%. While there are certainly more genes involved in these pathways, we chose these specific genes based upon previous literature suggesting that SNPs in these genes may impact the prostate cancer or other cancer phenotype and are also involved in oxidative stress response. Yet, evidence remains somewhat inconsistent and reported associations for single SNP analysis are weak.

Genotyping and SNP analyses

A total of 96 SNPs were genotyped and 21 of them were associated with the seven oxidative stress pathway genes (*MAPK14, NRF2, CAT, GPX1, GSTP1, SOD2,* and *XDH*). Additional SNPs in three genes (GSS, FXYD2, and CRYZ) were added based on known minor allele frequency (MAF) > 1%. All the SNPs were genotyped by Illumina BeadXpress VeraCode GoldenGate Genotyping Assay through the VeraCode technology (USA) by IGenix LLC according to the manufacturer's instructions. All SNPs were evaluated using Illumina Assay Design Tool and seven SNPs were removed based on poor cluster definition. The distribution of the genotypes polymorphism followed Hardy–Weinberg equilibrium. All the selected SNPs had call rates over 95%.

Statistical methods

Descriptive analyses on demographic characteristics of the participants were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with continuous variables expressed as mean (SD) and categorical variables expressed as number (%).

We conducted logistic regression to derive the odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of various oxidative stress-related pathways SNPs and the risk of prostate cancer using R statistical software. *p* values were obtained using score tests. Two subjects were removed who had PSA values > 100. The analysis was done for (a) prostate cancer as the phenotype of interest with biopsy-negative subjects as controls and (b) aggressive prostate cancer defined as Gleason score ≥ 7 as the phenotype of interest, while combining biopsy-negative subjects and those with Gleason score < 7 as controls. For either phenotype, the covariates we included were age, family history of prostate cancer, and PSA, which were chosen based on Bayesian information criterion (BIC).

We considered three types of genetic models: the common dominant models (one or two copies of a specific allele are required for a *x*-fold increase in prostate cancer risk), common recessive models (two copies of a specific allele are required for a *x*-fold increase in prostate cancer risk), and additive models (prostate cancer risk is increased by *x*-fold for genotype containing one copy of a specific allele and by 2*x*-fold for two copies of specific allele) into the analyses. For each type of genetic model, we reported the nominal *p* values as well as the Bonferroni-corrected *p* values adjusting for multiple comparisons.

Results

Participant characteristics

Table 1 describes the baseline characteristics of the total of 613 subjects with 231 prostate cancer subjects and 382 prostate biopsy-negative controls included in the current analysis. There was no statistically significant difference in baseline characteristics including BMI, race, marital status, smoking, alcohol, and total caloric intake. However, there was a significant difference in age and family history of prostate cancer, as cases were more likely to be older (65.40

Table 1 Basic characteristics of men in the genetic susceptibility, environment, and prostate cancer risk study

Participant characteristics	Prostate cancer cases ($n=231$)	Biopsy-negative controls ($n=382$)	p
	Mean (SD)	Mean (SD)	
Age, year	65.40 (6.78)	63.45 (5.75)	**0.0003**
BMI (kg/m^2) at baseline[a]	29.92 (5.25)	29.86 (5.54)	0.90
Height (cm) at baseline[a]	177.12 (6.88)	176.91 (6.54)	0.71
Prostate-specific antigen (PSA) at baseline (ng/mL)	17.22 (97.90)	5.78 (3.34)	**< 0.0001**
Prostate-specific antigen density (PSAD)	0.30 (0.80)	0.18 (0.80)	**< 0.0001**
Alcohol (g/day)[b]	22.73 (78.78)	14.83 (44.43)	0.21
Total energy intake (kcal/day)[b]	2,145.93 (1299.51)	2,100.13 (1096.35)	0.69
	n (%[c,d])	n (%[c,d])	
Race			0.59
White	215 (93.1)	351 (91.9)	
Non-White	16 (6.9)	31 (8.1)	
Family history of prostate cancer			0.05
Yes	57 (27.0)	71 (20.0)	
No	154 (73.0)	284 (80.0)	
Smoking[e]			0.81
Current	39 (17.41)	62 (16.99)	
Former	129 (57.59)	203 (55.62)	
Never	56 (25.00)	100 (27.40)	
Marital status[f]			0.73
Single, divorced, widowed	88 (39.82)	146 (40.33)	
Married/partner	133 (60.18)	216 (59.67)	

p-values less than 0.05 are bolded and are considered significant

t Tests were conducted for continuous variables (age, BMI, and height); nonparametric test was conducted for PSA and PSAD due to non-normal distribution; Chi square tests were conducted for categorical variables with expected cell frequencies ≥ 5; and Fisher's exact tests were conducted for categorical variables with expected cell frequencies < 5

[a]One subject in control group and two subjects in case group miss BMI and height information

[b]42 subjects in control group and 76 subjects in case group miss alcohol information and total energy intake

[c]For the categorical variables with missing values, χ^2 tests or Fisher's exact tests were conducted without including missing group

[d]Percentages may not add up to 100 due to rounding values

[e]17 subjects in control group and 7 subjects in case group miss smoking status information

[f]20 subjects in control group and 10 subjects in case group miss marital status information

Table 2 Proposed tagging SNPs for each gene of interest

MAPK14	NRF2	CAT	GPX1	GSTP1	SOD2	XDH
rs1775290	rs10506328	rs1049982	rs1050450	rs1079719	rs1799725	rs10190201
rs1380543		rs7104301	rs1800668	rs 947894	rs2842985	rs2281547
rs851024		rs511895		rs749174		rs494852
rs12665389		rs769217		rs8191443		rs6543628
rs13198204		rs1001179		rs1871041		rs185925
rs3804454						rs13418515
rs851006						rs12621192
						rs93088919

MAPK14 Mitogen-activated protein kinase-14 (p38); *NRF2* Nuclear factor erythroid 2–related factor 2; *CAT* Catalase; *GPX1* Glutathione1; *GSTP1* Glutathione-S-transferase P1; *SOD2* Superoxide dismutase; *XDH* Xanthine dehydrogenase

vs. 63.45 years old, $p = 0.003$) and have a family history of prostate cancer (27% vs. 20%, $p = 0.05$). Also, prostate cancer cases had much higher PSA and PSAD levels than biopsy-negative controls (Table 2).

Association of SNPs and prostate cancer as well as aggressive prostate cancer risk

The associations between SNPs in oxidative stress-related genes and overall prostate cancer risk are shown in Table 3. We found MAPK14 gene's SNP rs851023 was significantly associated with prostate cancer (OR 0.19, 95% CI 0.06–0.51, Padj = 0.015). The frequency of non-reference genotype (AA or GA) among cases was 93.4%, significantly lower than that among controls which was 98.7%. We did not find any of the other SNPs examined in association with prostate cancer. When comparing patients with aggressive prostate cancer (Gleason score ≥ 7) with biopsy-negative control, rs851023 was also found to be significantly associated with aggressive prostate cancer (OR 0.19, 95% CI 0.06–0.59, Padj = 0.047) (Table 4). However, when we compare patients with aggressive prostate cancer with non-aggressive prostate cancer (Gleason score < 7) and biopsy-negative control combined, the significance disappeared after multiple comparison adjustment (Table S1).

Discussion

In this study, we found the mitogen-activated protein kinases (MAPK)14 gene SNP rs851023 was significantly associated with prostate cancer risk. Compared to A/A genotype, men with G/G or G/A genotype in SNP rs851023 had an 81% decrease in the odds for overall prostate cancer. This association was also statistically significant for aggressive prostate cancer with Gleason score ≥ 7 as compared to biopsy-negative controls, but not significant when comparing less aggressive prostate cancer with Gleason score < 7 to biopsy-negative controls.

Given current understanding of the etiology of prostate cancer, there is a strong biologic rationale for the impact of aberrations in gene expressions and gene mutations associated with oxidative stress response and free radical detoxification pathways in cancer susceptibility [2]. Evidence regarding antioxidant dietary factors and antioxidant enzyme expression suggest that the cellular levels of reactive oxygen species (ROS) and other free radicals may be mechanistically involved, through their capacity to induce DNA damage, in prostate cancer initiation and progression. Thus, genes involved in the generation and detoxification of ROS and other free radicals and genes with roles in oxidative stress response are central to a multigenic model of susceptibility. Many of the proteins with roles in ROS detoxification have been identified and 15–20 of the genes have been systematically screened for common DNA sequence variants existing in the population. Similarly, many genes with key roles in the oxidative stress response signaling have been characterized and also screened for common sequence variants.

Prostate cancer is usually affected by multiple genes instead of single SNP [9, 10]. Previous studies have found many genetic polymorphisms related to prostate cancer, though only a few polymorphisms in the oxidative stress pathway-related genes have been investigated. A case–cohort study in the prospective Netherlands Cohort Study evaluated 14 genetic variants (CAT rs1001179, GPX1 rs17650792, GPX1 rs1800668, GPX1 rs3448, hOGG1 rs1052133, NOS2A rs2297518, NOS2A rs9282801, NOS3A rs1799983, NQ1 rs1800566, PON1 rs662, PON1 rs854560, SOD1 rs10432782, SOD2 rs4816407, and SOD2 rs4816407) in oxidative stress-related genes in association with advanced prostate cancer [11]. This study evaluated data from 952 cases and 1,798 subcohort subjects, showing that CAT rs1001179 was significantly associated with Stage III/IV and Stage IV prostate cancer risk. Our study was originally designed to also evaluate this CAT rs1001179 SNP but the data quality didn't meet our SNP inclusion criteria. A meta-analysis on catalase (CAT) C-262T polymorphism confirmed its association with prostate cancer with an OR 1.17 (95% CI 1.03–1.31) comparing TT to CT + CC genotypes [12]. In terms of the null finding from our study for other genes, our finding on *GSTP1* is consistent with an earlier meta-analysis showing *GSPT1*gene polymorphism was not associated with prostate cancer [13]. However, *GSTP1* methylation leading to underexpression of *GSTP1* may contribute to prostate cancer development [14]. For GPX1, we didn't evaluate rs1050450 due to low-quality data, but a meta-analysis has shown that GPX1 rs1050450 C>T polymorphism was not associated with the risk of prostate cancer but was associated with an increased risk of bladder cancer. [15]. Our null finding of GPX1 rs1800668 with either overall or aggressive prostate cancer is not consistent with a previous case–cohort study (the Netherlands Cohort Study) which found this SNP was associated with advanced (Stage III or IV) prostate cancer [16].For SOD2, we didn't find any associations for the 2 SNPs (rs1799725, rs2842985) examined, consistent with a meta-analysis showing SOD2 polymorphism was not associated with prostate cancer [17]. To our knowledge, we didn't find any studies that have examined NRF2 rs10506328 or the 8SNPs of XDH that we have examined which all had non-significant associations with prostate cancer in our study.

In our case–control study, we used non-invasive self-collected saliva samples to derive DNA for genotyping analysis. We only included six genes to reduce the SNP genotyping cost and used as few SNPs as possible for this association

Table 3 Distribution of cases and controls according to genotype frequencies and prostate cancer risk by selected susceptibility SNPs

SNP	Chr	Positions	Gene	Alleles[a,b]	Cases *N* (% of non-reference genotype[c])	Controls *N* (% of non-reference genotype[c])	Cases alternative allele freq %[d]	Controls alternative allele freq %[d]	Dominant model[e]			Recessive model[e]			Additive model[e]		
									OR	*p* value	Adjusted *p* value[f]	OR	*p*-value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]
rs851023	6	36006220	MAPK14	G/A	228 (93.4)	380 (98.7)	82	86	0.19 (0.06,0.51)	**0.001**	**0.015**	0.88 (0.6,1.29)	0.496	1	0.76 (0.55,1.04)	0.086	1
rs851024	6	35999564	MAPK14	C/G	222 (73.9)	375 (70.9)	48	46	1.23 (0.83,1.82)	0.304	1	1.17 (0.77,1.77)	0.463	1	1.15 (0.9,1.47)	0.276	1
rs12665389	6	36015094	MAPK14	G/A	224 (16.1)	379 (16.9)	8	9	1 (0.62,1.58)	0.995	1	1.03 (0.05,11.47)	0.983	1	1 (0.64,1.55)	0.998	1
rs13198204	6	56668855	MAPK14	C/A	227 (33)	382 (39)	18	21	0.77 (0.54,1.1)	0.156	1	0.88 (0.3,2.33)	0.803	1	0.81 (0.58,1.11)	0.186	1
rs3804454	6	36006998	MAPK14	T/G	228 (34.6)	380 (38.2)	20	21	0.93 (0.65,1.33)	0.699	1	2.43 (1.04,5.75)	0.035	0.774	1.06 (0.78,1.44)	0.691	1
rs851006	6	36065185	MAPK14	T/C	223 (93.3)	374 (96.3)	78	79	0.59 (0.27,1.3)	0.184	1	1.1 (0.77,1.58)	0.588	1	0.99 (0.74,1.34)	0.967	1
rs10506328	12	54687232	NRF2	A/C	225 (90.2)	379 (89.7)	67	68	1.21 (0.68,2.2)	0.519	1	1.03 (0.73,1.46)	0.866	1	1.06 (0.81,1.38)	0.67	1
rs1049982	11	34460541	CAT	T/C	224 (82.6)	377 (89.1)	64	65	0.5 (0.3,0.81)	0.005	0.099	1.14 (0.8,1.61)	0.468	1	0.89 (0.7,1.15)	0.377	1
rs7104301	11	34493638	CAT	A/G	224 (40.6)	375 (44)	24	25	0.83 (0.59,1.18)	0.31	1	1.32 (0.68,2.55)	0.404	1	0.94 (0.71,1.23)	0.644	1
rs511895	11	34487729	CAT	T/C	226 (83.6)	377 (83.8)	63	61	1.05 (0.66,1.69)	0.831	1	1.11 (0.78,1.58)	0.547	1	1.07 (0.84,1.36)	0.6	1
rs769217	11	34482908	CAT	C/T	218 (41.7)	369 (46.6)	25	25	0.84 (0.59,1.19)	0.33	1	2.26 (1.12,4.62)	0.02	0.444	1.02 (0.76,1.35)	0.902	1
rs1800668	3	49395757	GPX1	G/A	217 (53.5)	368 (51.4)	29	29	1.08 (0.76,1.54)	0.654	1	0.55 (0.24,1.16)	0.129	1	0.96 (0.72,1.28)	0.803	1
rs749174	11	67353253	GSTP1	G/A	227 (13.2)	378 (11.6)	13	11	1.06 (0.63,1.77)	0.831	1	1.06 (0.62,1.79)	0.836	1	1.03 (0.79,1.34)	0.832	1
rs2842985	6	160085434	SOD2	C/T	226 (93.8)	377 (96.3)	77	79	0.63 (0.28,1.43)	0.258	1	1.07 (0.75,1.53)	0.717	1	0.98 (0.73,1.33)	0.921	1
rs10190201	2	31585075	XDH	G/A	225 (72.4)	378 (75.9)	47	50	0.84 (0.57,1.25)	0.387	1	0.85 (0.56,1.27)	0.42	1	0.88 (0.69,1.12)	0.304	1
rs2281547	2	31598823	XDH	T/C	227 (59)	379 (61.2)	36	39	0.93 (0.66,1.33)	0.695	1	0.74 (0.45,1.2)	0.233	1	0.89 (0.7,1.14)	0.375	1
rs494852	2	31624836	XDH	C/T	221 (34.8)	375 (32.8)	20	18	1 (0.69,1.45)	0.979	1	1.14 (0.47,2.65)	0.766	1	1.02 (0.75,1.38)	0.899	1
rs6543628	2	31584839	XDH	G/A	221 (89.6)	368 (91)	71	73	0.83 (0.46,1.51)	0.533	1	0.83 (0.59,1.18)	0.306	1	0.87 (0.67,1.13)	0.292	1
rs185925	2	31609993	XDH	G/A	228 (85.1)	382 (88.7)	64	66	0.7 (0.42,1.16)	0.157	1	1.08 (0.76,1.52)	0.677	1	0.95 (0.74,1.22)	0.696	1
rs13418515	2	31612781	XDH	C/T	228 (32)	380 (26.6)	18	14	1.28 (0.88,1.85)	0.201	1	2.72 (0.85,9.4)	0.084	1	1.32 (0.94,1.84)	0.103	1

Table 3 (continued)

SNP	Chr	Positions	Gene	Alleles[a,b]	Cases *N* (% of non-reference genotype[c])	Controls *N* (% of non-reference genotype[c])	Cases alternative allele freq %[d]	Controls alternative allele freq %[d]	Dominant model[e]			Recessive model[e]			Additive model[e]		
									OR	*p* value	Adjusted *p* value[f]	OR	*p*-value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]
rs12621192	2	31588664	XDH	C/T	228 (52.2)	380 (52.6)	32	31	0.96 (0.68,1.35)	0.817	1	1.2 (0.69,2.07)	0.505	1	1.02 (0.79,1.31)	0.892	1

p-values less than 0.05 are bolded and are considered significant

[a]Major/minor alleles

[b]BB indicates homozygotes for the major allele, which were used as the reference group (shown in the original data files), AB indicates heterozygotes, and AA indicates homozygotes for the minor allele

[c]Reference genotype is AA, and non-reference genotypes are BB or AB. The % indicates the percentage of subjects having a non-reference genotype (BB or AB)

[d]The % of allele frequency for B is calculated as $(n2*1+n3*2)/(n1*2+n2*2+n3*2)$, if n1 stands for number of individuals with AA genotype, n2 stands for number of individuals with AB genotype, and n3 stands for number of individuals with BB genotype

[e]All models: logistic regression model was used to estimate OR (95% CI) of prostate cancer for each SNP adjusting for age, family history of prostate cancer, and PSA

[f]Bonferroni-corrected *p* values were calculated to control multiple comparisons

Table 4 Distribution of cases and controls according to genotype frequencies and aggressive prostate cancer risk by selected susceptibility SNPs (non-aggressive prostate cancer subjects are ignored, controls only include biopsy-negative subjects)

SNP	Chr	Positions	Gene	Alleles[a,b]	Cases *N* (% of non-reference genotype[c])	Controls *N* (% of non-reference genotype[c])	Cases alternative allele freq%[d]	Controls alternative allele freq%[d]	Dominant model[e]			Recessive model[e]			Additive model[e]		
									OR	*p* value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]
rs851023	6	36006220	MAPK14	G/A	155 (93.5)	380 (98.7)	0.83	0.86	0.19 (0.06,0.59)	**0.002**	**0.047**	0.99 (0.63,1.56)	0.961	1	0.82 (0.56,1.2)	0.304	1
rs851024	6	35999564	MAPK14	C/G	152 (75)	375 (70.9)	0.49	0.46	1.34 (0.86,2.14)	0.206	1	1.2 (0.74,1.93)	0.455	1	1.2 (0.9,1.6)	0.21	1
rs12665389	6	36015094	MAPK14	G/A	153 (15)	379 (16.9)	0.08	0.09	0.93 (0.53,1.59)	0.806	1	1.74 (0.08,19.27)	0.658	1	0.96 (0.56,1.59)	0.879	1
rs13198204	6	56668855	MAPK14	C/A	154 (35.1)	382 (39)	0.19	0.21	0.85 (0.56,1.29)	0.452	1	0.89 (0.24,2.65)	0.844	1	0.87 (0.6,1.25)	0.469	1
rs3804454	6	36006998	MAPK14	T/G	155 (33.5)	380 (38.2)	0.19	0.21	0.9 (0.59,1.35)	0.602	1	2.07 (0.73,5.55)	0.145	1	1 (0.7,1.43)	0.987	1
rs851006	6	36065185	MAPK14	T/C	150 (93.3)	374 (96.3)	0.78	0.79	0.54 (0.22,1.35)	0.166	1	1.01 (0.67,1.53)	0.97	1	0.92 (0.65,1.31)	0.641	1
rs10506328	12	54687232	NRF2	A/C	154 (89.6)	379 (89.7)	0.67	0.68	1.1 (0.58,2.19)	0.777	1	1.07 (0.72,1.59)	0.748	1	1.06 (0.78,1.44)	0.708	1
rs1049982	11	34460541	CAT	T/C	155 (81.3)	377 (89.1)	0.62	0.65	0.44 (0.26,0.77)	0.003	0.06	1.06 (0.71,1.58)	0.786	1	0.83 (0.62,1.1)	0.197	1

Table 4 (continued)

SNP	Chr	Positions	Gene	Alleles[a,b]	Cases *N* (% of non-reference genotype[c])	Controls *N* (% of non-reference genotype[c])	Cases alternative allele freq%[d]	Controls alternative allele freq%[d]	Dominant model[e]			Recessive model[e]			Additive model[e]		
									OR	*p* value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]	OR	*p* value	Adjusted *p* value[f]
rs7104301	11	34493638	CAT	A/G	153 (37.9)	375 (44)	0.23	0.25	0.71 (0.47,1.07)	0.101	1	1.32 (0.6,2.77)	0.47	1	0.84 (0.6,1.17)	0.313	1
rs511895	11	34487729	CAT	T/C	154 (82.5)	377 (83.8)	0.62	0.61	0.97 (0.58,1.66)	0.897	1	1.12 (0.75,1.68)	0.578	1	1.05 (0.79,1.38)	0.756	1
rs769217	11	34482908	CAT	C/T	146 (43.2)	369 (46.6)	0.27	0.25	0.9 (0.6,1.36)	0.62	1	2.83 (1.31,6.06)	0.006	0.123	1.12 (0.81,1.56)	0.485	1
rs1800668	3	49395757	GPX1	G/A	149 (51)	368 (51.4)	0.28	0.29	0.94 (0.62,1.41)	0.757	1	0.48 (0.16,1.18)	0.134	1	0.86 (0.61,1.2)	0.39	1
rs749174	11	67353253	GSTP1	G/A	155 (14.2)	378 (11.6)	0.14	0.11	1.16 (0.64,2.05)	0.615	1	1.2 (0.65,2.15)	0.542	1	1.09 (0.8,1.46)	0.575	1
rs2842985	6	160085434	SOD2	C/T	154 (93.5)	377 (96.3)	0.78	0.79	0.56 (0.23,1.45)	0.207	1	1.06 (0.7,1.6)	0.785	1	0.96 (0.68,1.37)	0.833	1
rs10190201	2	31585075	XDH	G/A	153 (72.5)	378 (75.9)	0.48	0.5	0.83 (0.53,1.31)	0.409	1	0.94 (0.59,1.49)	0.805	1	0.91 (0.68,1.21)	0.51	1
rs2281547	2	31598823	XDH	T/C	154 (55.2)	379 (61.2)	0.33	0.39	0.79 (0.53,1.18)	0.243	1	0.63 (0.33,1.13)	0.131	1	0.79 (0.59,1.05)	0.112	1
rs494852	2	31624836	XDH	C/T	152 (36.2)	375 (32.8)	0.21	0.18	1.03 (0.67,1.56)	0.89	1	1.08 (0.38,2.79)	0.877	1	1.03 (0.72,1.46)	0.865	1
rs6543628	2	31584839	XDH	G/A	149 (90.6)	368 (91)	0.71	0.73	0.91 (0.46,1.9)	0.793	1	0.82 (0.55,1.23)	0.336	1	0.87 (0.64,1.19)	0.393	1
rs185925	2	31609993	XDH	G/A	154 (85.7)	382 (88.7)	0.65	0.66	0.73 (0.41,1.32)	0.277	1	1.21 (0.81,1.81)	0.349	1	1.02 (0.76,1.38)	0.876	1
rs13418515	2	31612781	XDH	C/T	154 (33.8)	380 (26.6)	0.18	0.14	1.37 (0.89,2.09)	0.149	1	1.67 (0.33,7.16)	0.49	1	1.35 (0.91,1.98)	0.134	1
rs12621192	2	31588664	XDH	C/T	155 (52.3)	380 (52.6)	0.32	0.31	0.93 (0.62,1.38)	0.706	1	1.23 (0.65,2.26)	0.511	1	1 (0.74,1.35)	0.981	1

p-values less than 0.05 are bolded and are considered significant

[a]Major/minor alleles

[b]BB indicates homozygotes for the major allele, which were used as the reference group (shown in the original data files), AB indicates heterozygotes, and AA indicates homozygotes for the minor allele

[c]Reference genotype is AA, and non-reference genotypes are BB or AB

[d]All models: Logistic regression model was used to estimate OR (95% CI) of prostate cancer for each SNP adjusting for age, family history of prostate cancer, and PSA

[e]Bonferroni-corrected *p* values were calculated to control multiple comparisons

study. Therefore, our Tag SNP methods, which are a small subset of SNPs that is sufficient for performing association studies without losing the power of using all SNPs, balance the cost and the power. Tag SNP is sufficient to distinguish each pair of haplotype patterns in the haplotype block since the SNP within a haplotype block is inherited together.

To our knowledge, our study is the first reporting the association between rs851023 and cancer. Previously, there was one study reporting rs851023 in the p38 MAPK pathway that underlined susceptibility to impaired lung function when exposed to environmental tobacco smoke [18]. Oxidative stress caused by ROS activates the MAPK pathways [19]. Activation of MAPK signaling is involved in primary and metastatic prostate cancer [20]. MAPK includes three major kinases: p38, JNK, and ERK [21]. P38 family includes four isoforms: P38-α (MAPK11), P38-β (MAPK12), P38-δ (MAPK13), and P38-γ (MAPK14) [22]. The importance of the MAPK pathway in prostate cancer has been demonstrated through PC3 cells as well as prostate tissue [23, 24]: Huang et al. showed that p38 MAPK is necessary for TGF-β-mediated cell invasion in prostate cancer [23]; Ricote et al. showed that p38 MAPK transduction pathway was involved in benign prostate hyperplasia (BPH) as well as prostate cancer by enhancing cell proliferation and survival [24]. The results suggest an association between MAPK p38 and prostate cancer aggressiveness. While there are data describing polymorphisms in genes encoding for the enzyme products of the MAPK pathway (rs889312 and rs16886165 SNPs in *MAP3K1*) and breast cancer susceptibility [25], there are gaps in our knowledge regarding how polymorphisms in MAPK pathway may alter prostate cancer susceptibility including prostate cancer. Our finding on the association of *MAPK14* gene SNP rs851023 and prostate cancer in this study fills this gap.

Our study has many strengths. First, this is the first study to evaluate oxidative stress pathway genetic variability in association with total and high-grade prostate cancer. Second, our study population is more homogeneous. Participants were veterans with equal access to health care; therefore, confounding from healthcare access is limited. Third, our study provides important results on prostate cancer etiology with translational potential, especially on those lifestyle or environmental factors that are functional through oxidative stress pathway. Limitations are listed below. First, our findings may not be generalizable given the unique veteran population. Second, some of the important SNPs such as CAT rs1001179 fell out of our quality control and we were unable to evaluate them in this study.

In conclusion, this case–control study among a VA population showed an association with *MAPK14* gene SNP rs851023 polymorphism and prostate cancer. Our finding provides an approach for identifying individuals at high prostate cancer risk. How SNPs in the MAPK14 may interact with environmental and lifestyle factors that increase oxidative stress has not been evaluated and needs further investigation. Thus, well-designed longer-duration studies are needed to examine the complex associations and make preventive strategies to help high-risk individuals from prostate cancer development.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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