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Selenoprotein and antioxidant genes and the risk of high-grade prostate cancer and prostate cancer recurrence

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Abstract

Background—Observational studies suggest an inverse association between selenium and risk of prostate cancer. However, randomized controlled trials of selenium supplementation have reported conflicting results. Thus, we examined plasma selenium and selenium-related genes in relation to risk of high-grade prostate cancer and prostate cancer recurrence among men initially diagnosed with non-metastatic disease.

Methods—We measured plasma selenium and genotyped 73 single nucleotide polymorphisms in *TXNRD1*, *TXNRD2*, *GPX1*, *GPX3*, *GPX4*, *SEP15*, *SEPP1*, *SELENBP1*, *OGG1*, and *CAT* among 568 men with non-metastatic prostate cancer who underwent radical prostatectomy. We examined associations between plasma selenium, genotypes, and risk of high-grade prostate cancer (Gleason grade   8 or 7 with primary score   4; n=111) using logistic regression, and risk of prostate cancer recurrence (61 events; 3.8 y median follow-up) using Cox proportional hazards regression.

Results—Plasma selenium was not associated with risk of high-grade prostate cancer or prostate cancer recurrence. Less common alleles of rs11913319 in *TXNRD2* and rs125701 in *OGG1* were associated with an increased risk of high-grade prostate cancer. We observed associations between the risk of prostate cancer recurrence and multiple SNPs in *TXNRD1*, *TXNRD2*, *GPX3*, and *SEP15*. These associations were no longer statistically significant after adjustment for multiple comparisons.

Conclusions—Among men with non-metastatic prostate cancer, there is suggestive evidence that genetic variation in selenoproteins and related antioxidant enzymes may be associated with risk of high-grade disease at diagnosis and prostate cancer recurrence.

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Keywords

selenium; genetic polymorphisms; Gleason grade

Introduction

Selenium is an essential micronutrient involved in enzymatic antioxidant reactions and implicated in protection from prostate cancer, a major public health concern with 233,000 new cases predicted in the United States in 2014 (1). Circulating selenium levels and dietary selenium intake have both been associated with a reduced risk of prostate cancer in several meta-analyses of observational and randomized studies, with stronger associations with risk of advanced disease than total prostate cancer incidence (2–5). In a secondary analysis of the Nutritional Prevention of Cancer trial, daily supplementation with 200μg selenium in men with a history of skin cancer resulted in a 52% reduction in risk of prostate cancer (6). Conversely, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was terminated early after a median of 5.5 years due to concerns of increased risk of prostate cancer with vitamin E supplementation, increased risk of diabetes among men taking selenium, and no evidence of decreased risk of prostate cancer with either supplement (7). Further evaluation of the SELECT data demonstrated a statistically significant increased risk of high-grade prostate cancer among men with higher baseline selenium status randomized to selenium supplementation (8). The conflicting results of SELECT compared with previous studies could be attributed in part to the focus on total prostate cancer incidence as well as genetic differences between study populations in cellular pathways involving selenium.

In vivo, selenium is incorporated as part of the amino acid selenocysteine into the polypeptide chain of 17 known families of selenoproteins (9). Selenocysteine in the active site of the glutathione peroxidases (*GPX1-4*) and thioredoxin reductases (*TXNRD1* and *TXNRD2*) confers antioxidant activity (9). Selenoprotein P (SEPP1) transports selenium, and Sep 15 (*SEP15*) may regulate protein folding (9,10). Selenium-binding protein 1 (*SELENBP1*) functions in metabolism and protein transport (9). Recent studies have demonstrated associations between several prostate cancer outcomes and single nucleotide polymorphisms (SNPs) in the selenoprotein genes *GPX1-4*, *TXNRD1*, *SEP15*, and *SEPP1* (11–15). Additionally, the association between selenium and risk of incident or fatal prostate cancer may be modified by SNPs in *GPX1*, *TXNRD1-2*, *SEP15*, and *SEPP1* (13,15,16).

Candidate genes potentially involved in the relation between selenium and prostate cancer pathogenesis and progression include catalase (*CAT*), an antioxidant enzyme which has been associated with prostate cancer risk (17), and 8-oxoguanine glycosylase (*OGG1*), which repairs oxidative DNA damage and may modify the association between serum selenium and prostate cancer risk (18). *CAT* detoxifies superoxide and hydrogen peroxide in a common pathway with GPX and superoxide dismutase (SOD) enzyme isoforms, which have been well studied in prostate cancer in relation to selenium (19–27).

We previously reported that two SNPs in *SOD1* were inversely associated with risk of prostate cancer recurrence among men treated via radical prostatectomy for localized disease; SNPs in *SOD2* and *SOD3* were not associated with risk of high-grade prostate

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cancer or disease recurrence (25). To better understand the relation between genetic variability in selenoproteins and related antioxidant enzymes and prostate cancer progression, this study investigated SNPs in the candidate genes *TXNRD1*, *TXNRD2*, *GPX1*, *GPX3*, *GPX4*, *SEP15*, *SEPP1*, *SELENBP1*, *OGG1*, and *CAT* in relation to high-grade prostate cancer and prostate cancer recurrence among men who had localized disease treated with radical prostatectomy. We focused on the outcome of prostate cancer progression among men diagnosed with localized disease, because this outcome has clear clinical significance in a heavily screened population. Additionally, we investigated whether SNPs in these genes modified the association between plasma selenium concentration and risk of high-grade prostate cancer and prostate cancer recurrence. Due to our previous report of an association between SNPs in *SOD1* and prostate cancer recurrence (25) and prior reports of an interaction between SNPs in *SOD2*, selenium levels, and prostate cancer, we also examined whether SNPs in *SOD1* or *SOD2* modified associations between plasma selenium and high-grade prostate cancer or disease recurrence.

Materials and Methods

Study population

This study was conducted among men (n=1134) initially diagnosed with non-metastatic prostate cancer between 2000–2007 who underwent radical prostatectomy as primary treatment at UCSF. Participants were consented and provided fasting blood samples and residual tissue for research. We excluded men who had neoadjuvant treatment (e.g. hormones), men who did not consent to clinical follow-up, and men with missing clinical data (e.g. pre-surgical prostate specific antigen (PSA), Gleason score, stage), leaving 1003 eligible for analyses. Due to budgetary restrictions, we selected 700 of the 1003 men who met these criteria. We preferentially selected men with high Gleason grade prostate cancer (Gleason sum 8 or 7 with major Gleason score 4) to enable us to identify risk factors for aggressive disease; 32% of the men in our study population had high-risk disease vs. 22% of all men who underwent radical prostatectomy at UCSF between 2000–2007. Of the 700 patients selected for this study, 568 had sufficient DNA and plasma available for analysis. The median time from diagnosis to radical prostatectomy/date of blood draw for circulating selenium measurement and genomic DNA genotyping was 3.6 months and the median time from diagnosis to disease recurrence was 3.7 years. This study was approved by the Institutional Review Board of UCSF.

Circulating selenium measurement

Samples were centrifuged at 1900 RPM for 20 minutes at room temperature within two hours of blood draw. Plasma was aspirated into 500μl aliquots stored at −80°C. Selenium concentrations were assessed using flameless atomic absorption spectrometry (Perkin Elmer) with Zeeman background correction at the Fred Hutchinson Cancer Research Center (Seattle, WA) according to a standard protocol (28–31). The assay was calibrated using AA Winlab Method of Additions Calibration and three-point selenium standards in 0.2 % nitric acid. The standard curve was accepted when the R square is 0.995. Quality control samples were run before and after each batch of 10 study samples and sample concentrations were the average of duplicate measurements with a CV less than 10%.

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Genomic DNA and genotyping

Peripheral blood was collected using BD CPT Vacutainers Cell Preparation Tubes with Sodium Heparin (BD, Franklin Lakes, New Jersey, USA). The purification of buffy coat was carried out within two hours of blood draw. Each tube was centrifuged for 20 minutes at 1720 × g at room temperature, the upper plasma layer was discarded and the lymphocyte and monocyte band transferred into a 15 ml falcon tube using a sterile transfer pipette. Ten ml of PBS (phosphate buffered saline) were added and the tubes were centrifuged for 15 minutes at 300 × g. The supernatant was discarded and the cell sediment again re-suspended in 15 ml PBS and centrifuged (10 minutes, 300 × g). After discarding the supernatant, the remaining cell pellet was re-suspended in 1.8 ml cell preservation medium (10 % DMSO, 10 fetal calf serum, 80 DMEM) and stored at –80°C until high molecular weight DNA isolation. High molecular weight genomic DNA was extracted using a QIAamp DNA blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions using ddH_20 to elute DNA from the column. DNA concentration and quality were evaluated measuring the absorption ratio at 260/280 nm and 260/230 nm using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, USA) and standard agarose gel electrophoresis. The samples were diluted to 10 ng/ul for genotyping using the Sequenom MassARRAY system. Tag SNPs were selected using the HapMap database to characterize variation within each gene (+/– 5 kilobases), identifying variants with a frequency of at least 5 percent. Among SNPs in linkage disequilibrium (R^2 >0.8), we selected SNPs for analysis based on relevant function or previous literature.

Outcome assessment

The primary outcomes of interest were high-grade prostate cancer and prostate cancer recurrence. High-grade prostate cancer was defined as pathologic Gleason sum 8 or 7 with major Gleason score 4. Sensitivity analyses were also conducted using only Gleason sum 8 as the outcome. Prostate cancer recurrence was defined as two or more consecutive PSA values >0.2 ng/ml more than eight weeks after radical prostatectomy, initiation of secondary treatment six or more months after surgery, metastases to bone, or death due to prostate cancer. Recurrence was assessed by medical chart review.

Clinical and covariate data

Data on age, race, treatment, biopsy Gleason sum, stage, and prostate specific antigen (PSA) were abstracted from medical records. Prognostic risk score was calculated according to modified D'Amico criteria (Low: PSA <10 ng/ml and Gleason sum <7 AND T-stage T2a; Intermediate: PSA 10.1–20 ng/ml or Gleason sum = 7 OR T-stage = T2b-c; High: PSA >20 ng/ml or Gleason sum >7 OR T-stage T3a) (32). We also calculated the CAPRA risk score, a clinically relevant and validated composite risk score, based on Gleason grade, PSA at diagnosis, clinical T-stage, surgical margins, extra-capsular extension, seminal vesicle invasion, and lymph node invasion (33,34).

Statistical analysis

We analyzed circulating selenium concentration as well as SNPs in the candidate genes *TXNRD1*, *TXNRD2*, *GPX1*, *GPX3*, *GPX4*, *SEP15*, *SEPP1*, *SELENBP1*, *OGG1*, and *CAT* in

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relation to high-grade prostate cancer and prostate cancer recurrence. We also investigated whether SNPs in the genes listed above modify the relation between plasma selenium and high-grade prostate cancer or prostate cancer recurrence. In this analysis, we also included SNPs in *SOD1* and *SOD2*, for which associations with prostate cancer grade and recurrence in this cohort independent of circulating selenium have been previously published by our group (25).

To examine circulating selenium levels and SNPs in relation to risk of high-grade prostate cancer, we used a logistic regression model adjusted for age at diagnosis (continuous) and race/ethnicity (Caucasian vs. Non-Caucasian). Selenium levels were categorized into quartiles and modeled using indicator variables with the lowest quartile as the reference category. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to assess the magnitude and direction of the associations. To test for evidence of a linear trend across selenium levels, we modeled the median of each quartile as a continuous ordinal variable and used a Wald test to determine the *p*-trend. We modeled the SNPs using additive models (an ordinal variable was used indicating the number of less common alleles = 0,1,2) and co-dominant models (indicator variables were used with the homozygous common allele as the reference), and used Wald tests to calculate the *p*-value for the additive models. We collapsed the heterozygote and homozygous less common allele categories of SNPs when less than 5% of the study population was homozygous for the less common allele. In addition, we created a cross-product term between the circulating selenium levels (dichotomized at the median) and the SNPs (additive model) and used a Wald test to test for evidence of an interaction.

To examine the selenium levels and SNPs in relation to risk of prostate cancer recurrence, we used a Cox proportional hazards regression adjusting for age at diagnosis (continuous), race/ethnicity (Caucasian v. non-Caucasian), and prognostic risk score (D'Amico criteria: low, intermediate, high). Person-time was calculated from date of surgery to date of recurrence, death from another cause, or January 2014, whichever came first. As above, we modeled quartiles of selenium levels using indicator variables and tested for evidence of a linear trend by modeling the median of each quartile as a continuous term. We also examined the SNPs in relation to risk of recurrence using additive and co-dominant models.

Our study population was 87% Caucasian, 2% Black, 4% Asian, 6% other. To assess the potential of bias due to population stratification, we performed sensitivity analyses restricting to Caucasians. Analyses were conducted using SAS version 9.2 (SAS Institute Inc.) and two sided P values <0.05 were considered statistically significant. We adjusted for multiple comparisons using a Bonferroni correction for both model types and outcomes. The threshold for statistical significance was $0.05/73=0.0006$ for additive models and $0.05/146=0.0003$ for co-dominant models.

Results

Demographic characteristics of the study population, overall and by extreme quartiles of plasma selenium concentration, are listed in Table 1. The study population was 87% Caucasian men, and primarily composed of men with an intermediate prognostic risk score

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and Gleason score of 7. Plasma selenium concentration was not associated with risk of high-grade prostate cancer (Gleason grade 8 or 7 with primary score 4) or prostate cancer recurrence (Table 2).

High-Grade Prostate Cancer

Of 73 SNPs analyzed in 10 genes, we observed two SNPs nominally associated with high-grade prostate cancer (Figure 1, Table S1). In *TXNRD2*, the less common homozygote allele in SNP rs11913319 was associated with an increased risk of high-grade prostate cancer (OR: 2.01; 95% CI: 1.05, 3.84; *p*-value: 0.03) compared with the common homozygote allele. No association with high-grade prostate cancer was observed in the additive model for *TXNRD2*. We also observed a suggestive association between SNP rs125601 in the gene *OGG1* and high-grade prostate cancer (G>A: OR: 1.72; 95%CI: 1.11, 2.67; *p*-value: 0.02).

Prostate Cancer Recurrence

We observed associations with prostate cancer recurrence for SNPs in *TXNRD1*, *TXNRD2*, *GPX3*, and *SEP15* (Figure 2, Table 3). SNPs rs11610799 and rs7138318 in *TXNRD1* were both associated with an increased risk of prostate cancer recurrence (G>C: HR: 1.95; 95% CI: 1.03, 3.69; *p*-value: 0.04; and T>C: HR: 1.46; 95% CI: 1.04, 2.07; *p*-value: 0.03; respectively). In the co-dominant model, the heterozygous allele of SNP rs7138318 was associated with an increased risk of prostate cancer recurrence compared to the common homozygote allele (HR: 2.26; 95% CI: 1.31, 3.91; *p*-value: 0.004). A non-significant trend towards increased risk of prostate cancer recurrence was observed among patients homozygous for the less common allele (HR: 1.63; 95% CI: 0.69, 3.84; *p*-value: 0.27), however, few patients demonstrated this genotype (n = 58). The heterozygous allele of rs7488680 in *TXNRD1* was also associated with an increased risk of prostate cancer recurrence compared to the common homozygous allele in the co-dominant model (HR: 1.99; 95% CI: 1.10, 3.61; *p*-value: 0.02), but we observed no association with the homozygous less common allele or in the additive model.

Among SNPs in *TXNRD2*, we observed an increased risk of recurrence in additive models with the less common alleles in rs3788317 and rs599245 (G>T: HR = 1.51, 95%CI: 1.02, 2.24; *p*-value: 0.04; and T>G: HR = 1.83, 95%CI: 1.11, 3.02; *p*-value: 0.02). The heterozygous allele in rs3788317 was also associated with an increased risk of recurrence compared to the common homozygous allele in the co-dominant model (HR = 1.89, 95%CI: 1.13, 3.16; *p*-value: 0.01), while the homozygous less common allele showed a non-significant trend in the same direction (n = 30; HR = 1.44, 95%CI: 0.43, 4.74; *p*-value: 0.55). The heterozygous allele in rs1548357 was associated with a reduced risk of recurrence compared to the common homozygous allele (HR = 0.58, 95%CI: 0.34, 1.00; *p*-value: 0.05), and the less common homozygous allele trended in the same direction without reaching significance in both the co-dominant and additive models.

In *GPX3*, an increased risk of recurrence was observed in the additive model for the less common alleles of rs4958872 and rs8177426 (T>C: HR = 1.42, 95%CI: 1.00, 2.02; *p*-value: 0.05; and G>A: HR = 1.51, 95%CI: 1.05, 2.19; *p*-value: 0.03). For both SNPs, we observed a non-significant trend in the same direction for the heterozygote and less common

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homozygote allele compared to the common homozygote allele. We also observed a reduced risk of prostate cancer recurrence for the heterozygous allele at rs3763009 compared to the common homozygous allele (HR = 0.58, 95%CI: 0.33, 1.00; *p*-value: 0.05), while the less common homozygous allele trended in the same direction without reaching significance in the co-dominant and additive models.

We analyzed five SNPs in *SEP15*, and we observed an increased risk of prostate cancer recurrence among patients with the less common allele in rs1407131 and a decreased risk associated with the less common allele in rs527281 (T>C: HR = 1.90, 95%CI: 1.15, 3.15; *p*-value: 0.01; and C>G: HR = 0.36, 95%CI: 0.13, 0.99; *p*-value: 0.05). Note that all of the results for high-grade prostate cancer and prostate cancer recurrence were only suggestive of associations as none of them met the Bonferroni cutoff for statistical significance.

Interactions between Plasma Selenium and Genotypes

We then investigated how genotype influenced the association between plasma selenium concentration and high-grade prostate cancer as well as risk of recurrence. We selected four SNPs *a priori* based on existing literature for which we hypothesized interactions with selenium concentration (rs10432782 in *SOD1*, rs4880 in *SOD2*, rs2758330 in *SOD2*, rs1050450 in *GPX1*), all of which were null in this analysis. We then conducted an exploratory analysis of all SNPs analyzed in this study for interactions with plasma selenium concentration in association with tumor grade or recurrence. We identified two SNPs with p-interaction<0.01, both in the gene *OGG1* (Table 4). For both rs1052133 and rs2304277, plasma selenium was associated with an increased risk of high-grade prostate cancer in individuals with the less common homozygous genotype, whereas, in individuals with the common homozygous genotype, plasma selenium was associated with a non-significant trend of decreased risk of high-grade disease.

Discussion

In this case-only study, we examined the relationship between common polymorphisms in candidate genes of selenoproteins and other antioxidant enzymes with risk of high-grade prostate cancer and prostate cancer recurrence in men initially diagnosed with localized disease. Plasma selenium was not associated with high-grade prostate cancer or risk of prostate cancer recurrence in our study. However, we did observe associations between high-grade prostate cancer and SNPs in *TXNRD2* and *OGG1* as well as associations between risk of prostate cancer recurrence and SNPs in *TXNRD1*, *TXNRD2*, *GPX3*, and *SEP15*. No associations remained significant after adjustment for multiple comparisons, and our results must be interpreted with caution due to limited sample size. However, previously reported associations with prostate cancer risk or mortality for some of the SNPs studied suggest a reduced likelihood of false positive results.

TXNRD1 and *TXNRD2* are selenoprotein genes involved in the regulation of reduction-oxidation signaling and have been investigated as pharmacologic therapeutic targets in prostate cancer (35,36). The less common allele of rs5992495 in *TXNRD2*, which was associated with increased risk of prostate cancer recurrence in our study, encodes a serine to arginine amino acid substitution. Laboratory studies have yet to examine the functional

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consequence of this mutation, however, substitution of an amino acid with opposing charge is likely to result in altered function. No other study has reported an association between this SNP and prostate cancer, although, past studies have reported associations between SNPs in *TXNRD1* and *TXNRD2* and prostate cancer- specific mortality as well as interactions with plasma selenium concentration and prostate cancer risk (12,16). Two of the SNPs in *TXNRD1* we found to be associated with prostate cancer recurrence are in complete linkage disequilibrium (r^2=1.0) with two SNPs reported to be significantly associated with prostate cancer mortality (rs10778322 with rs7138318 and rs4964785 with rs7488680) in a case-control analysis (12). In that study, the heterozygotes of both SNPs were associated with a reduced risk of prostate cancer mortality compared to the homozygote common allele (rs10778322: HR = 0.57, 95% CI: 0.35, 0.96; rs4964785: HR = 0.55, 95% CI: 0.33, 0.91). Although the heterozygote alleles were associated with an increased risk of prostate cancer recurrence in our study, we do not expect these associations to be identical to this case-control study design because our case-only analysis does not have the same healthy comparison group. These four SNPs are located outside of the coding regions of the gene, and their mechanistic relation to gene function or expression is not known.

Glutathione peroxidases catalyze the reduction of hydrogen peroxide and are implicated in the development of numerous cancers (9). Members of the GPX family are expressed in both the cytosol (*GPX1*) and extracellular plasma (*GPX3*) (9). Prior work has identified an association between the less common allele of rs8177447 in *GPX3* and increased odds of regional/distant stage prostate cancer (12). We investigated rs8177426, which is in linkage disequilibrium with rs8177447 (r^2=0.95), and found an increased risk of prostate cancer recurrence after radical prostatectomy associated with the less common allele, supporting an association with extraprostatic spread. Since both SNPs are located outside of gene coding regions, the underlying mechanism is remains unclear. Additionally, SNP rs3448 in *GPX1*, which has a previously reported association with prostate cancer risk, was borderline-associated with high-grade prostate cancer in our study (12).

SEP15 is highly expressed in the prostate and likely regulates protein folding (9,37). One previous study reported an association between the less common allele of rs1407131 in *SEP15* and prostate cancer specific mortality (A>G: HR = 2.85, 95%CI: 1.45, 5.59) (15). Notably, we observed an association between rs1407131 and an increased risk of prostate cancer recurrence, supporting a role of *SEP15*, and possibly this SNP, in prostate cancer progression. In two previous studies, rs561104 in *SEP15* was associated with prostate cancer specific mortality and risk of localized prostate cancer (12,15), but we observed no association with high-grade prostate cancer or recurrence.

We also investigated *OGG1*, which repairs free radical-induced DNA damage independently of selenium. Previous work identified an association between the less common allele in rs125701 and a reduced risk of prostate cancer in men with high serum selenium levels (18). In our study population, the less common allele in this SNP was associated with an increased risk of high-grade prostate cancer. Our exploratory analysis of the interaction between SNPs in *OGG1* and plasma selenium also revealed a previously unreported increased risk of high-grade prostate cancer associated with the less common homozygote genotype in *OGG1* SNPs rs1052133 and rs2304277 in men with high plasma selenium

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levels. These data combined warrant further examination of the role of *OGG1* in prostate cancer progression, particularly in men with high selenium levels.

This study has several limitations. First, we observed few recurrence outcomes due to short follow-up and had a limited sample size to test for evidence of interactions. Second, plasma selenium was measured only at a single time point, however, a single measurement of plasma selenium has been shown to provide a reasonable estimation of long term dietary intake (38). Third, this analysis was conducted among primarily Caucasian men, thus our results may not be generalizable to populations with different race/ethnicity distributions. Fourth, we were limited to the available data on possible confounders, which did not include smoking, dietary, or physical activity data. However, the relationships observed were for germline genetic variants, which are unlikely to be confounded by lifestyle factors. We acknowledge the possibility that observed associations may be due to linkage disequilibrium with genetic variants that were not measured as well as the potential for false positives due to multiple testing.

Overall, we have replicated previously described associations between prostate cancer and SNPs in *TXNRD1*, *GPX3*, *SEP15*, and *OGG1*. We have also identified additional associations not previously described in the literature, including an association between prostate cancer recurrence and a missense mutation in *TXNRD2*. As a result of multiple testing, our results must be interpreted with caution, particularly for SNPs with no previously reported associations with prostate cancer. Associations we observed for SNPs with a previously known association with prostate cancer are less likely to be falsely positive and reinforce the importance of these genes in the biology of prostate cancer. Furthermore, the generalizability of these findings, now in multiple populations, suggests genotyping of these SNPs may have clinical utility in determining prognosis or altering diagnostic or therapeutic recommendations. Future research is needed to assess the utility of these genetic variants to provide prognostic information and to investigate the underlying biology of the relationships observed. Furthermore, an improved understanding of selenoprotein genotype may aid in the interpretation of results from selenium supplementation trials such as SELECT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Plot of log-transformed p-values for an additive model of association between genotype at each of 73 SNPs in candidate antioxidant and selenoprotein genes and the relative risk of high-grade prostate cancer at diagnosis. The horizontal line indicates a chosen significance threshold of P=.05, and colors indicate gene as shown.

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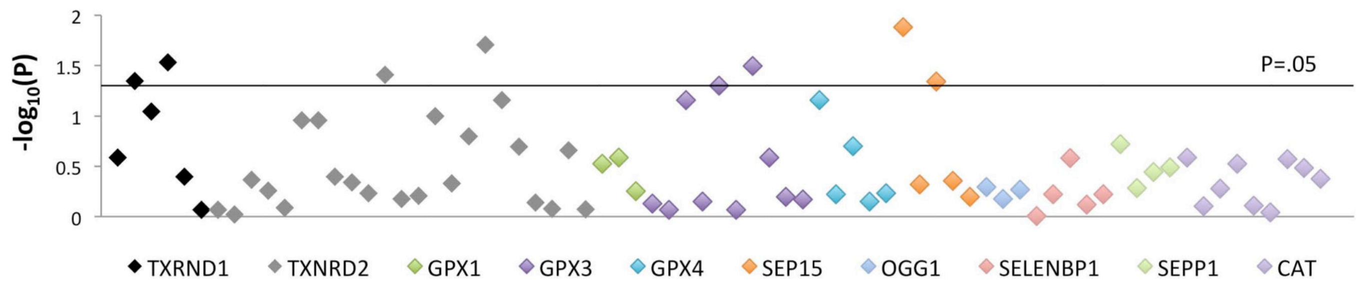

Figure 2.

Plot of log-transformed p-values for an additive model of association between genotype at each of 73 SNPs in candidate antioxidant and selenoprotein genes and the relative risk of prostate cancer recurrence. The horizontal line indicates a chosen significance threshold of P=.05, and colors indicate gene as shown.

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Table 1

Descriptive statistics of 568 men initially diagnosed with non-metastatic prostate cancer, overall and by extreme quartiles of plasma selenium.

	Total	Extreme quartiles of plasma selenium	
		Q1	**Q4**
Number of participants	568	137	140
Age at diagnosis, y, mean ±SD		59.0±7.0	58.6±6.4
PSA at diagnosis, ng/ml, mean ±SD		7.5±5.8	6.9±5.4
Plasma selenium, μg/ml, median (range)		101.0 (26–108)	139.4 (128–232)
Caucasian, % (N)	87 (495)	90	85
Clinical Stage, % (N)			
T2b or less	26 (145)	28	33
T2c	52 (296)	52	48
T3a or higher	22 (127)	20	19
Gleason Score, % (N)			
<7	36 (203)	42	34
7 (3+4)	43 (247)	39	49
7 (4+3)	14 (77)	12	12
>7	7 (41)	7	5
Prognostic Risk Group[a], % (N)			
Low	11 (60)	12	13
Intermediate	57 (325)	58	57
High	32 (182)	30	30
CAPRA, % (N)			
<3 (Low)	31 (178)	35	30
3–5 (Intermediate)	26 (145)	25	28
>5 (High)	5 (29)	4	3

[a]Based on the modified D'Amico criteria (Low= Gleason sum <7, PSA at diagnosis <10 ng/ml, AND clinical T-stage=T2a or less; Intermediate= Gleason sum <7, PSA at diagnosis = 10–19.9 ng/ml, AND clinical T-stage <T3a OR Gleason sum =7, PSA at diagnosis <20 ng/ml, AND clinical T-stage <T3a; High= Gleason sum >7, PSA at diagnosis >= 20ng/ml, OR clinical T-stage = T3a or higher)

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Table 2

Relative risk of high-grade prostate cancer and prostate cancer recurrence among 568 men initially diagnosed with non-metastatic disease, by plasma selenium concentration.

		High-grade[a]			Recurrence			
Quartile of Plasma Se	**Plasma Se µg/mL, median (range)**	**No. of Events**	**Multivariate OR (95% CI)[b]**	**P-trend[c]**	**No. of Events**	**Person-years**	**Multivariate HR (95% CI)[d]**	**P-trend[c]**
1	101.0 (26–108)	26	1.0 (ref.)		19	530	1.0 (ref.)	
2	112.4 (108–117)	26	0.79 (0.44–1.43)		14	555	0.70 (0.36–1.38)	
3	122.9 (117–128)	36	1.26 (0.72–2.21)		16	541	0.84 (0.44–1.61)	
4	139.4 (128–232)	23	0.82 (0.45–1.51)	0.84	12	534	0.71 (0.35–1.43)	0.35

[a]Total Gleason 8 or Gleason=7 with primary score 4

[b]Multivariate logistic regression model adjusted for age at diagnosis (years), serum PSA at diagnosis, stage at diagnosis

[c]P-trend calculated by modeling the median of each quartile as a continuous term

[d]Multivariate hazard ratio adjusted for age at diagnosis (years) and prognostic risk group (low, intermediate, high)

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Table 3

Relative risk of prostate cancer recurrence among a cohort of 568 men initially diagnosed with non-metastatic disease using both co-dominant and additive models for selected SNPs with p 0.05[a]

Gene/SNP	MAF[b]	Allele[c]	Co-dominant HR (95% CI)[d]	P-trend	Additive HR (95% CI)[d]	P-value
TXRND1						
rs11610799	0.07	GG (n=487)	1.0 (ref)		1.0 (ref)	
		GC/CC (n=80)			1.95 (1.03–3.69)	0.04
rs7138318	0.31	TT (n=272)	1.0 (ref)		1.0 (ref)	
		CT (n=235)	2.26 (1.31–3.91)	0.004	1.46 (1.04–2.07)	0.03
		CC (n=58)	1.63 (0.69–3.84)	0.27		
rs7488680	0.42	TT (n=192)	1.0 (ref)		1.0 (ref)	
		GT (n=279)	1.99 (1.10–3.61)	0.02	1.16 (0.82–1.65)	0.40
		GG (n=97)	1.11 (0.47–2.64)	0.81		
TXRND2						
rs1548357	0.30	TT (n=279)	1.0 (ref)		1.0 (ref)	
		CT (n=239)	0.58 (0.34–1.00)	0.05	0.71 (0.47–1.08)	0.11
		CC (n=49)	0.72 (0.28–1.86)	0.50		
rs3788317	0.24	GG (n=325)	1.0 (ref)		1.0 (ref)	
		GT (n=210)	1.89 (1.13–3.16)	0.01	1.51 (1.02–2.24)	0.04
		TT (n=30)	1.44 (0.43–4.74)	0.55		
rs5992495	0.18	TT (n=380)	1.0 (ref)		1.0 (ref)	
		GT/GG (n=188)			1.83 (1.11–3.02)	0.02
GPX3						
rs3763009	0.33	CC (n=259)	1.0 (ref)		1.0 (ref)	
		TC (n=236)	0.58 (0.33–1.00)	0.05	0.69 (0.46–1.03)	0.07
		TT (n=69)	0.61 (0.26–1.46)	0.27		
rs4958872	0.28	TT (n=303)	1.0 (ref)		1.0 (ref)	
		TC (n=212)	1.43 (0.84–2.44)	0.19	1.42 (1.00–2.02)	0.05
		CC (n=51)	2.00 (0.94–4.27)	0.07		

Gene/SNP	MAF[b]	Allele[c]	Co-dominant HR (95% CI)[d]	P-trend	Additive HR (95% CI)[d]	P-value
TXRND1						
rs8177426	0.20	GG (n=374)	1.0 (ref)		1.0 (ref)	
		GA (n=161)	1.61 (0.94–2.73)	0.08	1.51 (1.05–2.19)	0.03
		AA (n=30)	2.14 (0.89–5.14)	0.09		
SEP15						
rs1407131	0.14	TT (n=417)	1.0 (ref)		1.0 (ref)	
		CT/CC (n=150)			1.90 (1.15–3.15)	0.01
rs527281	0.07	CC (n=485)	1.0 (ref)		1.0 (ref)	
		GC/GG (n=77)			0.36 (0.13–0.99)	0.05

[a] See Table S2 for results from all SNPs analyzed

[b] Minor Allele Frequency (MAF) calculated using allelic distribution of the study population

[c] Heterozygote combined with homozygous less common genotype when less common homozygote less than 5% of cohort

[d] Survival model adjusted for age at diagnosis (years), prognostic risk score (Modified D’Amico criteria: low, intermediate, high)

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Table 4

Relative risk of high-grade prostate cancer by quartile of plasma selenium, stratified by genotype for selected SNPs with p-interaction 0.05[a]

SNP (gene)	**Allele**	**Quartile of selenium (μg/ml)**				**P-value**
		Q1	**Q2**	**Q3**	**Q4**	
rs1052133 (OGG1)	CC (n=301)	1.0 (ref.)	0.85 (0.42–1.74)	0.74 (0.35–1.57)	0.42 (0.18–1.00)	0.15
p-int=0.006	GC (n=227)	1.0 (ref.)	0.63 (0.22–1.84)	2.19 (0.91–5.26)	0.91 (0.35–2.34)	0.67
	GG (n=38)	1.0 (ref.)	5.66 (0.19–165.2)	9.19 (0.36–232.7)	26.51 (0.93–753.3)	0.03
rs2304277 (OGG1)	GG (n=321)	1.0 (ref.)	0.75 (0.37–1.52)	0.73 (0.36–1.50)	0.39 (0.17–0.93)	0.12
p-int=0.005	AG (n=209)	1.0 (ref.)	0.97 (0.34–2.78)	2.62 (1.04–6.60)	0.99 (0.37–2.68)	0.66
	AA (n=35)	1.0 (ref.)	2.40 (0.06–91.1)	5.23 (0.16–171.0)	15.84 (0.96–545.9)	0.03

[a]Multivariate model adjusted for age at diagnosis (years).

[b]P-interaction created from modeling a cross-product term between circulating selenium levels and individual SNPs and Wald test was used to test for evidence of an interaction