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Association of genetic variations of selenoprotein genes, plasma selenium levels and prostate cancer aggressiveness at diagnosis

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Abstract

Background—Genetic variations in some of the selenoprotein genes, alone or together with an individual's selenium status, may influence risk or progression of prostate cancer. We investigated the impact of genetic variants of selenoproteins on plasma selenium levels and cancer aggressiveness at diagnosis in men with localized prostate cancer (PCa).

Methods—The study cohort comprised 722 patients seen at Dana-Farber Cancer Institute who had localized/locally advanced PCa (i.e. stage T3 or less, N0 and M0) from 1994 to 2001. 55 Tagging single nucleotide polymorphisms (SNPs) from 6 selenoprotein genes (*TXNRD1, TXNRD2, SEP15, GPX3, SELENBP1 and SEPP1*) were analyzed. Logistic regression is used to examine associations of genotypes and plasma selenium levels with risk of aggressive disease, defined as D'Amico intermediate/high risk categories. Step down permutation was applied to adjust for multiple comparisons.

Results—348 patients (48%) had aggressive disease at diagnosis. Two SNPs were associated with cancer aggressiveness at diagnosis (unadjusted P= 0.017 and 0.018, respectively). The odds ratio for aggressive disease in patients carrying *TXNRD2* rs1005873-AG/GG genotypes or *SELENBP1* rs10788804-AG/AA genotypes was 1.54 (95% CI=1.08, 2.20) and 1.45 (95% CI=1.07, 1.98), respectively, compared to *TXNRD2* rs1005873-AA or *SELENBP1* rs10788804-GG carriers. Four SNPs in *TXNRD2* (rs1005873, rs13054371, rs3788310, and rs9606174) and the

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rs230820 in *SEPP1* were associated with plasma selenium levels (unadjusted $P<0.05$). Permutation adjusted p-values were not statistically significant for all these comparisons at the cut-off point of 0.05.

Conclusion—We identified polymorphisms in selenoproteins that may influence the plasma selenium levels and may be associated with the risk of presenting with aggressive PCa in men with localized or locally advanced PCa. These results should be validated in other independent datasets.

Keywords

prostate cancer; selenium; selenoproteins

Introduction

Several prospective and case-control studies have shown that selenium may act as a chemopreventive agent in prostate cancer (PCa), potentially through inhibition of cellular proliferation, apoptosis, antiangiogenesis, or antioxidant pathways (1–5). After 13 years' follow-up, the Nutritional Prevention of Cancer Trial revealed a 50% decrease in PCa incidence among those randomized to 200 μg of selenium per day (6, 7). However, the larger Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed that neither selenium nor vitamin E, alone or in combination, was effective for the primary prevention of PCa among men with oral supplementation with L-selenomethionine 200 μg/day (8–11). Of note, the great majority of diagnoses in SELECT were localized low to intermediate grade prostate cancer, and it remains unknown whether having higher selenium levels affects the risk of more aggressive disease or disease progression in men who already have PCa. Similarly, little is known about how genetics may modify associations between selenium and aggressive PCa or disease progression. Our previous reports highlighted the potential influence of genetic polymorphisms in selenium-related antioxidant genes, alone or in combination with circulating selenium levels, on PCa progression (12–15). It was suggested that selenium intervention for PCa may still be important for men of specific genotypes or with specific tumor phenotypes.

Selenium can incorporate into the polypeptide chain as part of the amino acid selenocysteine, and proteins that contain selenocysteine are defined as selenoproteins (16). Selenoproteins are present in all lineages of life. In humans, there are 17 selenoprotein families (16, 17). Some selenoproteins have been functionally characterized and correlated to cancer development and/or progression, including thioredoxin reductases *(TXNRDs*), glutathione peroxidases (*GPX*s), 15-kDa selenoprotein (*SEP15*), selenoprotein P (*SEPP1*) and selenium binding protein 1 (*SELENBP1*) (18–25). *TXNRD1* and *TXNRD2*, which are involved in antioxidant defense through the thioredoxin redox cycle, are not only responsible for numerous cytoplasmic functions, but also play a role in control of cell growth in which the redox function is essential for growth stimulation and apoptosis (18, 21). The levels of *TXNRD1* and *TXNRD2* in tumor cells is often much higher than those in normal tissues and tumor proliferation seems to be crucially dependent on an active thioredoxin system (26). Among all *GPXs, GPX3*, which was frequently deleted or methylated in PCa, can suppress PCa growth and metastasis through down-regulating the expression of c-Met (24). Expression of *SEPP1* is also dramatically reduced in a subset of human prostate tumors,

mouse tumors, and in the androgen-dependent (LNCaP) and androgen-independent (PC-3) PCa cell lines, suggesting its role in PCa development (10). *SELENBP1* is deregulated in both colorectal and ovarian cancers, and such decreased gene expression could promote cancer cell proliferation (23, 26).

Single nucleotide polymorphisms (SNPs) in some of the selenoprotein genes, alone or together with the selenium status, may influence risk or progression of cancers including PCa (14–15, 27–30). In *TXNRD2* gene, rs4485648, rs1548357, rs5748469 (A66S) and rs756661 were either significantly or marginally associated with breast cancer risk in a case-control study containing 4474 cases and 4,580 controls ($P \leq 0.05$) (27). A SNP in the 3′ untranslated region of *SEP15* (rs5859) was found to decrease the efficiency of the Sec insertion at higher concentrations of selenium (31), which modified the association between selenium status and lung cancer risk in smokers (32). Additionally, three *SEP15* genetic variants (rs479341, rs1407131 and rs561104) were significantly associated with PCa-specific mortality in the Physicians' Health Study (14). The rs561104 of *SEP15* significantly modified the association of plasma selenium with PCa survival (14), suggesting the important role of *SEP15* genetic polymorphisms in disease development of PCa. Additionally, SNPs in *SEPP1* may influence risk of colorectal cancer (30).

We hypothesized that genetic variants in selenoprotein genes may influence plasma selenium levels as well as the risk of aggressive PCa at diagnosis. Based on the function and cellular distribution, we selected 6 selenoprotein genes, *GPX3, SELENBP1, SEP15, SEPP1, TXNRD1* and *TXNRD2*, which are potentially relevant to PCa and performed a comprehensive investigation of the correlation of their genetic variants with disease aggressiveness in a cohort consisting of patients with localized PCa. Selenoproteins *GPX1* and *GPX4* have previously been investigated in the same study cohort and no association with PCa aggressiveness was found (13). Thus these two genes are not included in this study.

Patients and Methods

Study cohort

The study cohort was identified from the Prostate Clinical Research Information System (CRIS) at the Dana-Farber Cancer Institute and has been previously described (12, 13). The cohort is comprised of 778 patients who were diagnosed with localized/locally advanced PCa (i.e. stage T3 or less, N0 and M0) between January 1994 and March 2001 and who consented and donated blood for research before undergoing any type of local therapy. Of these patients, 722 men with complete clinical and genomic data were included in the current study. Plasma selenium measures were available in a subset of 486 men. This study was approved by the DFCI institutional review board.

SNP selection and Genotyping

As previously described (33), tagging SNPs for *TXNRD1, TXNRD2, SEP15, GPX3, SELENBP1* and *SEPP1* genes were selected, based on the HapMap phase II data of Utah residents with Northern and Western European ancestry (CEU) population. The tagging

SNPs were selected by pairwise algorithm implemented in the Haploview 4.1 program (http://www.broad.mit.edu/mpg/haploview) to capture the unmeasured variants $r^2 > 0.8$. In total, 64 SNPs were genotyped, which capture all common variations among the CEU population of all gene loci. Genomic DNA was prepared from blood using QIAamp DNA Blood mini kit (Qiagen Inc, Valencia, CA, USA). Genotyping was done using Sequenom iPLEX matrix-assisted laser desorption/ionization–time of flight mass spectrometry technology at the core facility of Boston Children Hospital. Nine SNPs were removed from analysis because of their poor genotype rate (<85%) or failed Hardy Weinberg equilibrium (*p-value*<0.001). The final analysis contained 55 SNPs: 6 SNPs in *TXNRD1*, 24 SNPs in *TXNRD2*, 5 SNPs in *SEP15*, 11 SNPs in *GPX3*, 5 SNPs in *SELENBP1*, and 4 SNPs in *SEPP1*.

Statistical Analysis

The primary outcome of interest was presentation of aggressive PCa at diagnosis, defined as stage T2b-T3, or PSA > 10ng/mL or biopsy Gleason ≥ 7 (D'Amico intermediate/high risk categories; 34). Non-aggressive cases therefore were stage <T2b, PSA≤10ng/ml, and Gleason <7. SNPs were analyzed under a co-dominant model (i.e. three genotype groups with the homozygous common allele as the reference) as well as a dominant model where rare homozygote was combined with heterozygote at each locus. Associations of disease aggressiveness with genotypes were evaluated using a chi-square test or Cochran-Armitage trend test; odds ratio (OR) and 95% confidence intervals (CI) were reported. Plasma selenium levels were analyzed as continuous values as well as five ordered groups (categorized according to the quintile cut-off values (108,3, 118.0, 125.5, 139.7 ug/L, respectively; equivalent to 1.08, 1.18, 1.26, 1.40 ppm). Wilcoxon rank-sum test and Cochran-Armitage trend test were conducted to compare selenium levels between genotypes. Multivariable logistic regression was undertaken to evaluate the association of selenoprotein genotypes with disease aggressiveness after adjusting for plasma selenium levels and age at diagnosis (continuous) or test for interaction between genotypes and selenium levels on disease aggressiveness.

To account for multiple testing, we performed a step-down permutation procedure, developed by Westfall and Young (35). 5000 permutation data sets were generated by randomly shuffling patients' outcomes (for example, aggressive vs. non-aggressive cancer). From each simulation dataset, we computed *P* value (for the statistical test described above) and then calculated the successive minima of new *P* values. The permutation adjusted *P* value is defined as the proportion of re-sampled data sets where the minimum pseudo *P* value is less than or equal to the original *P* value. Permutation tests were conducted using R2.8.0 (https://www.r-project.org/); all other statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). All *P* values are two sided.

Results

Patient and disease characteristics at diagnosis

The detailed patient demographics and clinical characteristics at diagnosis of men with localized/locally advanced PCa have been previously described (12, 13). Briefly, 722

patients were included in the current analysis. Patients' median age was 62 years (interquartile range (IQR): 56–68), the majority (96%) self identified as Caucasian, and their median PSA level was 6.3 ng/mL (IQR: 4.8–9.3). Nearly half of the cohort (48%) had aggressive disease at diagnosis (defined above as being D'Amico intermediate or high risk).

Association of plasma selenium levels with aggressive PCa

Four hundred eighty six men had selenium measures available. The demographic and clinical characteristics of these 486 men were comparable to those of the whole cohort (data not shown). The median selenium level in this population was 121.4 ug/L (range: 64.2–221.1, IQR: 110.3–135.4); higher selenium levels were associated with slightly higher risk of aggressive disease at diagnosis (p-trend= 0.05, Table 1).

Association of genetic variations of selenoproteins with aggressive PCa

In total, 55 SNPs covering 6 gene loci (*GPX3, SELENBP1, SEP15, SEPP1, TXNRD1* and *TXNRD2*) were analyzed (Supplemental Table 1). Two polymorphisms, rs1005873 (in *TXNRD2*) and rs10788804 (in *SELENBP1*), were associated with increased risk of developing aggressive disease in univariate analysis (P-value <0.05, Table 2). In a co-dominant model, the ORs for aggressive disease in patients carrying *TXNRD2* rs1005873-AG and -GG genotypes are 1.44 (95% CI= 1.00, 2.08) and 3.22 (95% CI= 1.01, 10.2), respectively, compared to the rs1005873-AA carriers (*P-value* = 0.007). For rs10788804 in *SELENBP1*, AG and AA genotypes had a borderline association with increased risk of aggressive disease (OR = 1.46, 95% CI= 1.05, 2.02; and OR = 1.45, 95% CI= 0.92, 2.27, respectively), compared to the GG carriers (*P-value* = 0.042). In a dominant model, the rs1005873-AG/GG and the rs10788804-AG/AA genotypes are also positively correlated with the risk of aggressive disease at diagnosis (OR = 1.54, 95% CI= 1.08, 2.20, *P-value* = 0.017; OR = 1.45, 95% CI= 1.07, 1.98, *P-value* = 0.018, respectively).

We also observed an additive effect between *TXNRD2* rs1005873 and *SELENBP1* rs10788804 polymorphisms. Patients were grouped according to the number of favorable genotypes (AA for the rs1005873 and GG for the rs10788804) each individual carried. Decreasing the number of favorable genotypes statistically significantly increased the risk of developing aggressive PCa (*P-value* = 0.002; Table 2). Individuals with zero or one favorable genotype had an OR of 2.08 (95% CI= 1.28, 3.38) and 1.56 (95% CI= 1.10, 2.21), respectively, for presenting with aggressive disease compared with those with two favorable genotypes (Table 2).

In summary, our data suggested that *TXNRD2*-rs1005873 and *SELENBP1*-rs10788804 may be associated with presenting with aggressive disease at diagnosis. However, after 5000 permutation analysis accounting for multiple comparisons (as we were testing 55 SNPs from 6 genes), the association of rs1005873 and rs10788804 with aggressive disease was not statistically significant (permutation adjusted *P-value*= 0.52 and 0.54, respectively).

Association of selenoprotein genotypes with plasma selenium levels

We further addressed the potential influence of genetic variants of selenoproteins on plasma selenium levels in this study cohort. Four SNPs in *TXNRD2* (rs1005873, rs13054371,

rs3788310, and rs9606174) and one SNP in *SEPP1* (rs230820) were associated with plasma selenium levels in univariate analysis (*P-values* <0.05; Table 3). Results were similar if selenium levels were analyzed as continuous values or as the ordered quintile groups. It is also noted that patients with *TXNRD2* rs1005873 risk genotypes (AG/GG) had a higher plasma selenium level than that of rs1005873-AA carriers (125.0 vs. 120.9, *P-value* = 0.002), in accordance with the trend of higher risk of aggressive disease for higher selenium levels. *TXNRD2* rs9606174 and rs1005873 variants were in almost complete Linkage Disequilibrium (LD) (r^2 = 0.92). The rs3788310 was also in high LD with rs9606174 and rs1005873 variants (r^2 = 0.58 and 0.54, respectively). However, LD between rs13054371 and other 3 SNPs were minimal ($r^2 \leq 0.05$). All these associations became insignificant in the permutation test accounting for multiple comparisons (permutation adjusted *P-value* > 0.05). The associations of plasma selenium levels with other selenoprotein SNPs are summarized in supplemental table 1.

Association of TXNRD2 and SELENBP1 variants with aggressive PCa after adjusting for plasma selenium levels

As mentioned above, our data showed that *TXNRD2* rs1005873 might be associated with both disease aggressiveness and plasma selenium levels. We also observed the correlation of higher selenium levels with increased risk of aggressive disease in this cohort. Therefore, it is possible that *TXNRD2* variants may correlate with disease aggressiveness through its effect on selenium levels. After adjusting for age and plasma selenium quintile levels in a multivariable model, the association of the *TXNRD2* rs1005873 with disease aggressiveness remained statistically borderline significant (adjusted OR= 1.55, 95% CI=1.01, 2.39, p-value= 0.047, Table 4). Similarly, *SELENBP1* rs10788804, which was not correlated with plasma selenium level, retained its association with disease aggressiveness at diagnosis after adjustment for selenium levels (adjusted OR= 1.47, 95% CI=1.00, 2.15, *p*= 0.048, Table 4). Results were similar for the additive effect of rs1005873 and rs10788804 in the multivariable analysis (Table 4), or if selenium levels were analyzed as continuous values (data not shown). The selenium levels were no longer statistically significantly associated with risk of aggressive cancer in the multivariable analysis when adjusted for age and genetic variants at *TXNRD2*-rs1005873, or *SELENBP1*-rs10788804, or in the additive model combining the two loci (*p-trend* =0.218, 0.064 and 0.250, respectively). The test for interaction between selenium levels and gene variants on risk of aggressive cancer was also null (p-interaction>0.05, data not shown), indicating none of these selenoprotein SNPs modified the effect of selenium levels on cancer aggressiveness.

Discussion

The association of selenium levels with PCa risk has been controversial (6–11). Since selenium is incorporated into a number of antioxidant proteins or selenoproteins and is required for their redox function, we hypothesized that the impact of selenium on PCa may be mediated by functional variants in antioxidant metabolizing genes. We tested the impact of SNPs in six different selenoproteins on the risk of having aggressive PCa at diagnosis and the plasma selenium levels in a large retrospective series of patients with localized or locally advanced PCa. We observed that two SNPs (*TXNRD2*-rs1005873 and *SELENBP1*-

rs10788804) were related to the risk of aggressive PCa at diagnosis, defined as D'Amico intermediate/high risk categories of stage T2b-T3, or PSA > 10 ng/mL or biopsy Gleason ≥ 7, with a nominal p-value less than 0.05. Interestingly, SNPs in *TXNRD2* were also associated with plasma selenium level. Patients carrying the risk allele of *TXNRD2* rs1005873 had a higher level of plasma selenium. After adjusting for plasma selenium levels, the association of rs1005873 with disease aggressiveness remained, though it was borderline statistically significant, which suggested that there might be intricate relationships between rs1005873, plasma selenium level, and risk of aggressive disease. *TXNRD2*, encoding a selenocysteine containing protein, is a member of pyridine nucleotide-disulfide oxidoreductases and is a key enzyme in the regulation of the intracellular redox environment. The rs1005873 is located in an intron of *TXNRD2*. The exact mechanistic mechanism explaining the correlation of the rs1005873 with plasma selenium level is not known. Further studies are needed to validate these findings and to explore functionality.

SELENBP1 is a selenium-binding protein and its exact biological function is unclear. However, it was suggested that *SELENBP1* expression may be an important predictor of response to chemoprevention or chemosensitization with certain forms of selenium in esophageal tissues (36). We observed that the rs10788804 in *SELENBP1* was correlated with the risk of aggressive cancer at diagnosis. However, *SELENBP1* had no impact on plasma selenium levels in our cohort. rs10788804 is an intronic SNP in *SELENBP1* and the molecular detail involved in the association of rs10788804 with PCa risk remains to be determined. *SEPP1,* a plasma selenoprotein, might be responsible for some of the extracellular antioxidant defense or might be involved in the transport of selenium (20). A SNP in *SEPP1* was found to have a marginal correlation with plasma selenium level though genetic variants of *SEPP1* had no association with aggressive PCa risk in this study.

Genetic variants in *SEP15* were previously reported to be statistically significantly associated with the time from diagnosis to PCa-specific death and rs561104 in *SEP15* significantly modified the effect of plasma selenium on survival in the Physicians' Health Study (14). SEP15-rs1407131 or -rs527281 were also found to relate with prostate cancer recurrence in men who underwent radical prostatectomy (15). In our cohort, we did not observe an association of genetic variants of *SEP15* with disease aggressiveness at diagnosis or with plasma selenium levels. We had insufficient data to study the association of the above factors with disease recurrence or survival in our cohort.

We observed a slightly higher risk of aggressive disease compared to non-aggressive disease for higher levels of selenium in the study population (p-trend=0.053). However, this association was insignificant in multivariable analysis after adjusting for age and TXNRD2-rs1005873 or SELENBP1-rs10788804 variants. Selenium level was not correlated with age in this cohort (Spearman rank correlation = −0.067, p-value=0.139), therefore, the observed effect of selenium levels on cancer aggressiveness may have been partially confounded by these genetic variants. The role of selenium in preventing PCa remains controversial in the literature (6–11). Recent work from the Health Professionals Follow-Up Study suggested that selenium supplementation may adversely increase risk of PCa mortality after diagnosis of nonmetastatic PCa. These conflicting findings highlight the importance of further

exploring the candidate genes potentially involved in the relation between selenium and prostate cancer aggressiveness (37).

We noticed that after 5000 permutation analyses adjusting for multiple comparisons, none of the results were statistically significant. Nevertheless, this study identified genetic variants in selenoproteins that might potentially impact the aggressiveness of the disease at diagnosis or the selenium level in plasma. Genotyping of these SNPs may have potential clinical utility in evaluating aggressive versus nonaggressive disease in patients diagnosed with localized PCa. Future work is needed to confirm these findings in independent cohorts and to investigate the underlying biology of the observed relationship.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

PCa	prostate cancer
SNP	single nucleotide polymorphism
TXNRD	thioredoxin reductases
GPX	glutathione peroxidase
SEP15	15-kDa selenoprotein
SEPP1	selenoprotein P
SELENBP1	selenium binding protein 1
OR	odds ratio
CI	confidence intervals

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Table 1

Risk of aggressive prostate cancer according to quintiles of plasma selenium level (N=486).

Selenium level	Risk of aggressive cancer*				Odds ratio (95% CI)	p-trend
	Non-aggressive		Aggressive			
	N	%	N	%		
I(low)	60	(61%)	39	(39%)	1.00(Reference)	0.053
II	60	(61%)	39	(39%)	1.00(0.57,1.77)	
III	55	(57%)	41	(43%)	1.15(0.65,2.03)	
IV	55	(57%)	41	(43%)	1.15(0.65,2.03)	
V(high)	45	(47%)	51	(53%)	1.74(0.99,3.08)	
I+II	120	(61%)	78	(39%)	1.00(Reference)	0.035
III+IV	110	(57%)	82	(43%)	1.15(0.77,1.72)	
V	45	(47%)	51	(53%)	1.74(1.07,2.85)	

*Aggressive disease was defined as stage T2b-T3, or PSA > 10ng/mL or biopsy Gleason ≥ 7 (D'Amico intermediate/high risk categories).

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Table 2

Risk of aggressive prostate cancer and its association with selenoprotein genotypes (N=722).

SNP	Risk of aggressive prostate cancer		Odds ratio (95% CI)		
	Non-aggressive	Aggressive	Co-dominant	Dominant	Permutation adjusted *P*-value
All patients (N=722)	374 (52%)	348 (48%)			
TXNRD2 (rs1005873)					
AA	290(54%)	248(46%)	1.00(Reference)	1.00(Reference)	
AG	65(45%)	80(55%)	1.44(1.00,2.08)	1.54(1.08,2.20) (AG+GG)	
GG	4(27%)	11(73%)	3.22(1.01,10.2)		
***P*-value** *			**0.007**	**0.017**	**0.523**
SELENBP1 (rs10788804)					
GG	145(58%)	106(42%)	1.00(Reference)	1.00(Reference)	
AG	172(49%)	183(51%)	1.46(1.05,2.02)	1.45(1.07,1.98) (AG+AA)	
AA	53(49%)	56(51%)	1.45(0.92,2.27)		
***P* -value** *			**0.042**	**0.018**	**0.544**
Number of favorable genotypes **					
2	116(60%)	76(40%)	1.00(Reference)		
1	196(49%)	200(51%)	1.56(1.10,2.21)		
0	44(42%)	60(58%)	2.08(1.28,3.38)		
***P*-value** *			**0.002**		

* trend test for a co-dominant model and chi-square test for a dominate model; Permutation adjusted P-value was based on the dominant model accounting for multiple comparisons as we were testing 55 SNPs from 6 genes.

** favorable genotype refers to AA at rs1005873 and GG at 10788804.

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Table 3

Association of selenoprotein genotypes with plasma selenium levels (N=486).

Genotype	N	Selenium value (ug/L)		Selenium quintile level (I:low; V:high) Odds ratio (95% CI)**				
		Median	*P*-value* (permutation-*P*)	II vs. I	III vs. I	IV vs. I	V vs. I	P-trend
SEPP1 rs230820								
CC	143	118.7	0.041(0.805)	1.0(Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)	
AC/AA	330	122.0		1.4(0.8,2.6)	1.4(0.8,2.5)	1.7 (0.9,3.1)	1.9 (1.0,3.6)	0.037
TXNRD2 rs13054371								
CC	129	117.6	0.005(0.192)	1.0(Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)	
CT/TT	347	122.4		0.8(0.4,1.4)	1.1(0.6,2.1)	1.7(0.9,3.2)	2.0(1.0,4.1)	0.004
rs3788310								
GG	393	121.0	0.027(0.674)	1.0(Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)	
AG/AA	85	124.0		1.8(0.8,4.0)	1.5(0.6,3.4)	2.0(0.9,4.4)	2.4(1.1,5.2)	0.041
rs9606174								
AA	364	120.8	0.007(0.274)	1.0(Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)	
AG/GG	118	124.0		1.6(0.8,3.1)	1.4(0.7,2.9)	1.4(0.7,2.8)	2.7(1.4,5.3)	0.011
rs1005873								
AA	355	120.9	0.002(0.104)	1.0(Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)	
AG/GG	112	125.0		1.3(0.6,2.7)	1.4(0.7,2.8)	1.4(0.7,2.9)	2.9(1.4,5.7)	0.003

*Wilcoxon rank-sum test and permutation adjusted *P*, accounting for multiple comparisons as we were testing 55 SNPs from 6 genes.

**Odds ratio for higher selenium quintile level estimated from Ordinal Logistic Regression.

Table 4

Association of selenoprotein genotypes with aggressive prostate cancer after adjusting for plasma selenium quintile levels and age at diagnosis in multivariable Logistic regression.

SNP	Odds ratio for aggressive disease* (95% CI)	*p*-value
TXNRD2 (rs1005873)		
AA	1.00 (reference)	
AG/GG	1.55(1.01, 2.39)	0.047
SELENBP1 (10788804)		
GG	1.00 (reference)	
AG/AA	1.47(1.00, 2.15)	0.048
Number of favorable genotype		
2	1.00 (reference)	
1	1.45(0.94, 2.25)	
0	2.18(1.20, 3.97)	0.034

* Estimated from 3 separate logisitic regression models, each including the analyzed SNP and simutaineously adjusted for the selenium quintile levels and age at diagnosis in the model. N=467, 481 and 463 respectively.