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Single-nucleotide polymorphisms within the antioxidant defence system and associations with aggressive prostate cancer

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Abstract

Objective—To study the effects of oxidative stress on prostate cancer development as the exact biological mechanisms behind the relationship remain uncertain. We previously reported a statistically significant interaction between circulating selenium levels, variants in the superoxide dismutase 2 gene (*SOD2*; rs4880), and risk of developing prostate cancer and presenting with aggressive prostate cancer.

Patients and methods—We genotyped men with localized/regional prostate cancer for 26 loci across eight genes that are central to cellular antioxidant defence: glutathione peroxidase (*GPX1, GPX4*), peroxisome proliferator-activated receptor γ coactivator (*PPARGC1A, PPARGC1B*), *SOD1, SOD2*, and *SOD3*, and 'X-ray repair complementing defective repair in Chinese hamster cell 1' (*XRCC1*). Among 489 men, we examined the relationships between genotypes, circulating selenium levels, and risk of presenting with aggressive prostate cancer at diagnosis, as defined by stage, grade and prostate-specific antigen (PSA) level (213 aggressive cases).

Results—Two variants in *SOD2* were significantly associated with the risk of aggressive prostate cancer (rs17884057, odds ratio 0.83, 95% confidence interval 0.70–0.99; and rs4816407, 1.27, 1.02–1.57); men with A alleles at rs2842958 in *SOD2* had lower plasma selenium levels (median 116 vs 121.8 μg/L, $P = 0.03$); and the association between plasma selenium levels and risk of aggressive prostate cancer was modified by *SOD1* (rs10432782) and *SOD2* (rs2758330).

Conclusion—While this study was cross-sectional and these associations might be due to chance, further research is warranted on the potential important role of antioxidant defence in prostate cancer.

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Keywords

single nucleotide polymorphisms; superoxide dismutase; glutathione peroxidase; aggressive prostate cancer; plasma selenium

Introduction

The association of oxidative stress with cancer development and progression has been studied for several decades, but many questions remain [1,2]. Oxidative stress is caused by reactive oxygen species (ROS) that are inadequately detoxified. Exogenous (consumed) antioxidants and endogenous (internally synthesized) antioxidants contribute to the 'antioxidant defence system' (ADS) [3,4]. Superoxide dismutases (SODs), as endogenous antioxidants, catalyse the breakdown of superoxide, a ROS, into oxygen and hydrogen peroxide, thus protecting the cell from superoxide toxicity. Humans have three forms of SOD; SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. A single-nucleotide polymorphism (SNP) in exon-2 of *SOD2* (rs4880) causes an amino-acid substitution (Ala16Val) [5]. The Val-variant causes partial arrest of the precursor protein within the inner membrane and decreases formation of the active *SOD2* tetramer [6]. The Ala-SOD2 has been linked with risk of Parkinson's disease [7], Alzheimer's disease [8], breast cancer [9,10], colorectal cancer [11], sporadic motor neurone disease [12], and severe alcoholic liver disease [13], whereas the Val-SOD2 has been reported as a risk factor for lung carcinoma [14] and dilated cardiomyopathy [15].

In a nested case-control study within the Physicians' Health Study (600 cases and 600 controls), we reported that although Ala16Val polymorphisms in *SOD2* rs4880 (hereafter referred to as AA, VA, or VV for brevity) alone were not a risk factor for prostate cancer, the individuals with AA with lower plasma antioxidants levels (either selenium alone or a combination of selenium, lycopene and vitamin E) had a significantly higher risk of prostate cancer (especially advanced prostate cancer) than either those with AA with higher plasma antioxidant levels or those with V alleles [16]. Subsequently, in a different set of patients with prostate cancer, there was a statistically significant interaction whereby men with the same AA *SOD2* variant and higher circulating selenium levels had a lower risk of presenting with aggressive prostate cancer, while men with a V allele in rs4880 and high selenium levels had a higher risk [17] than men with V alleles and low selenium levels.

Based on these data, we expanded our SNP selection to assess other variants in *SOD2* and other genes in the ADS in relation to prostate cancer status. We assessed SNPs on *SOD1*, *SOD2* and *SOD3*, glutathione peroxidase (*GPX1*, *GPX4*), peroxisome proliferators-activated receptor γ coactivator 1 (*PPARGC1α*, *PPARGC1β*), and X-ray repair complementing defective repair in Chinese hamster cells 1 (*XRCC1*). The biochemical function of GPX1 is to convert free hydrogen peroxide to water, and that of GPX4 (also known as phospholipid hydroperoxidase) is to convert lipid hydroperoxides to their corresponding alcohols. Both GPX1 and GPX4 contain selenium at their active site [18]. PPARGC1 regulates genes involved in energy metabolism, and is required for the induction of many ROS-detoxifying enzymes, including SOD2 and GPX1 [19]. XRCC1 protein is involved in the efficient repair of DNA damage, such as single-strand breaks, which are formed by exposure to ionizing radiation, alkylating agents, or ROS [20]. We hypothesized that variants in these genes would be associated with risk of aggressive prostate cancer, and might interact with circulating selenium to influence risk of aggressive prostate cancer in men with local/regional prostate cancer.

Patients and methods

Patients with prostate cancer for this study were selected from the Prostate Clinical Research Information System and Specimen Tracking Inventory Program databases at the Dana-Farber Cancer Institute [21]. The Prostate Clinical Research Information System is a central repository of patient data, including comprehensive follow-up of all patients. To be eligible for this study, patients had to have a diagnosis of localized/locally advanced prostate cancer (i.e. stage T3 or less, N0 and M0); consented and donated blood for research before undergoing any type of local therapy; and consented to be followed clinically for research purposes. Of 778 patients who fulfilled these study criteria, 753 were selected according to the availability of complete clinical data and genomic DNA. Plasma collected before any type of therapy was available for selenium assessment in 489 of the selected patients.

The prognostic risk at diagnosis was categorized using modified criteria of D'Amico *et al*. [22,23], as: low risk (≤ T2a and PSA level ≤ 10 ng/mL and Gleason sum ≤ 6); intermediate risk (T2b or PSA level 10–20 ng/mL, or Gleason sum 7); and high risk (> T2b or PSA level > 20 ng/mL or Gleason sum > 7). The primary outcome of interest was the presentation of aggressive prostate cancer at diagnosis, defined as stage T2b-T3, or PSA level > 10 ng/mL or biopsy Gleason 7 (corresponding to the intermediate-/high-risk categories).

Blood was withdrawn using EDTA as an anticoagulant and centrifuged at 1000 ***g*** for 10 min at 17 °C. Purified plasma was aliquoted and stored at − 80 °C until used for the analysis. This process was complete 2–16 h after sample withdrawal. Genomic DNA was prepared from EDTA-blood using the QIAamp DNA Blood mini kit (Qiagen Inc, Valencia, CA, USA) within 24 h after withdrawal and stored at 4 °C. DNA concentration was assayed using pico-green (Invitrogen, Carlsbad, CA, USA), and adjusted to 5 ng/μL for genotype analysis.

We initially identified 56 SNPs in *SOD1*, *SOD2* and *SOD3*, *GPX1*, *GPX4*, *PPARGC1α*, *PPARGC1β*, and *XRCC1*, derived from the National Cancer for Biotechnology Information database by heterozygosity ratio (≥ 0.05). Six SNPs that did not conform to Hardy–Weinberg equilibrium ($P < 0.01$) were removed. Any SNP with a minor allele frequency of < 5% (six) was also removed from analysis. Among the remaining 44 SNPs, tagging polymorphisms were selected using the Haploview procedure (http://www.broad.mit.edu/mpg/haploview/) by setting a pair-wise linkage disequilibrium (LD) mode to ($r^2 \geq 0.8$ and logarithm of odds, LOD, ≥ 3). In all, 26 SNPs that captured most of the haplotypes in a region of LD were selected and examined for their association with risk of aggressive prostate cancer.

Except for the GCG repeat and rs1050450 within *GPX1*, all other SNPs were analysed by sequential PCR-mass spectrometry systems (Sequenom, San Diego, CA, USA) 'Increase Plexing Efficiency and Flexibility for MassARRAY System Assay or homogeneous Mass EXTENDED assay'. Methods for assessing the variants in GPX1 are described below.

The GCG repeat SNP within *GPX1* was analysed using a previously described procedure [24] with minor modifications. Genomic DNA (40 ng) was amplified using 17 pmol each of primers (forward: 5′*FAM*-GAAACTGCCTGTGCCACGTGACC-3′ and reverse: 5′-CGAGAAGGCATACACCGACTGGGC-3′) in 22 μL PCR buffer (Qiagen) containing 1.5 mM $MgCl_2$, 1.8 mM dNTP, Q solution, 1.5 units of Taq polymerase (all Qiagen). The PCR reaction had an initial denaturing temperature at 94 °C (2 min) followed by 35 cycles of denaturing (94 °C; 30 s), annealing (62.5 °C; 1 min), and extension (72 °C; 30 s) steps. An 8-min extension at 72 °C followed the final cycle. The 1 μl of PCR product was diluted with water to 50 μL; 2 μL of diluted PCR product were mixed with 10 μL formamide, 0.25 μL Gene Scan-500 LIZ Size Standard (Applied Biosystems, Foster City, CA, USA), and water

to adjust the final volume to 20 μL. This mixture was applied to a POP-7 capillary array which was linked to an automated fluorescence detection system, ABI 3730 (Applied Biosystems). Using 'Genemapper Software v4.0' and 'Peak Scanner Software v1.0' for the analysis, the GCG repeat number was calculated as ((fragment length – 154 bp)/3 = $(GCG)_n$). This equation was confirmed by sequencing 30 PCR samples.

For rs1050450 in *GPX1*, a SNP (CCC/Pro or CTC/Leu) within *GPX1* locates in exon-2 at the amino-acid position between 198 and 200. Shifts in amino-acid position depend on the number of GCG repeats (4–6x) in exon-1. Consequently, this SNP is referred either as Pro198Leu or Pro200Leu. This was analysed using a previously described procedure [25] with minor modifications. Genomic DNA (20 ng) was amplified using 12.5 pmol each of primers (forward: 5′-CTACGCAGGTACAGCCGCCGCT-3′ and reverse: 5′-CAGGTGTTCCTCCCTCGTAGGT-3′) in 12.5 μL 60 mM Tris-HCl (pH 9.5) buffer containing 15 mM ammonium sulphate, 2 mM $MgCl_2$, 1.6 mM dNTP, and 0.6 units platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA). The PCR had an initial denaturing temperature at 94 °C (2 min) followed by 35 cycles of denaturing (94 °C; 30 s), annealing (62.5 °C; 1 min), and extension (72 °C; 30 s) steps. An 8-min extension at 72 °C followed the final cycle. 7.5 μL of PCR product were digested by incubating with 25 units of ApaI (New England BioLabs, Beverly, MA, USA) at 25 °C for 2 h. Digested products were visualized on a 2% agarose gel stained with ethidium bromide. Fragment patterns specific for three genotypes were: Pro/Pro (CCC/CCC; 118 bp, 74 bp), Pro/Leu (CCC/CTC; 192 bp, 118 bp, 74 bp), and Leu/Leu (CTC/CTC; 192 bp).

Plasma selenium level was analysed using a previously described procedure in the laboratory of Irena King (Fred Hutchinson Cancer Research Center [26]). Diluted 99 : 300 in 0.5% Triton X-100, plasma selenium concentration (μg/L) was analysed by flame-less atomic absorption (Perkin-Elmer 5000; Perkin Elmer Corp., Norwalk, CT, USA) using an electrode-less discharge lamp operating at λ = 196.0 nm and a 1′Vov platform graphite furnace. Twenty-seven of 489 plasma samples were analysed in duplicate with 'blind' numbering; the median coefficient of variation was within 5.3%. For these samples, the first measurement was used in the analysis.

Patient disease characteristics at diagnosis were summarized as counts and percentages, or as median (range), and interquartile range of levels. Plasma selenium levels between genotypes were compared using the Wilcoxon rank-sum test. Associations of disease aggressiveness with genotypes were evaluated using a chisquare test. Relative risk (RR) and 95% CI compared to common homozygote were estimated using a generalized linear model for binomial data with a log-link rather than a logit-link function. Associations of disease aggressiveness with selenium levels were evaluated using a Cochran-Armitage test for trend, where selenium levels were categorized to five ordered groups according to the quintile thresholds (108.3, 118.0, 125.5, 139.7 μg/L, respectively; equivalent to 1.08, 1.18, 1.26, 1.40 ppm). The likelihood ratio test from the generalized linear model was used to test for an interaction between genotypes and selenium levels on disease aggressiveness, where selenium levels were evaluated both as quintile groups and continuous values. All analyses were conducted with $P < 0.05$ (two-sided) considered to indicate statistical significance.

Results

The demographic and clinical characteristics at diagnosis of the selected 753 men with prostate cancer are summarized in Table 1. The patients were mostly white, with a median age of 62 years and a median PSA level of 6.3 ng/mL. About half of the patients had low-risk disease, a third had intermediate-risk disease, and ≈ 10% had high-risk disease. Age was not associated with the risk of aggressive disease (data not shown). Among these 753

men, 489 had plasma selenium levels assessed with a median of 121.4 μg/L. The demographic and clinical characteristics of these 489 men were comparable to those of the 753 men (data not shown).

We assessed 24 SNPs, one triplet (GCG) repeat polymorphism within *GPX1*, and one deletion/insertion polymorphism of three nucleotides (AGA) within *SOD1*. Also, 18 SNPs that were captured by one of the 26 listed SNPs or rs4880 [17] are shown in Table 2.

Table 3 shows: the genotype distribution of each SNP, and association of each SNP with the risk of aggressive prostate cancer (753 total, 359 with aggressive disease); plasma selenium levels in allele groups of each SNP (489 patients); and the interaction between genotypes, selenium level, and risk of aggressive prostate cancer (489 patients, 213 with aggressive disease).

We combined rare homozygotes (frequency < 0.05) with heterozygotes. Consequently, 16 SNPs were analysed between two genotypes, and nine with three genotypes. GCG repeats within *GPX1* showed six genotypes with combinations of 4–6 repeats. Genotype distributions in this group were comparable to those reported in other Caucasian or global cohorts (see the National Cancer for Biotechnology Information database).

Two SNPs (rs17884057 and rs4816407) within *SOD1* were associated with the risk of aggressive prostate cancer at borderline significance ($P = 0.04$ and 0.05, respectively, Table 3). Men with (–)(–) or (–)(AGA) alleles at the rs17884057 locus had a lower risk of aggressive disease than men with (AGA)(AGA) alleles (RR 0.83, 95% CI 0.70–0.99). Also, men with AG or GG alleles at the rs4816407 locus had a higher risk of aggressive disease than men with AA alleles (RR 1.27, 95% CI 1.02–1.57). No other SNP was significantly associated with the risk of aggressive prostate cancer (Table 3).

Comparison of selenium levels among genotype groups of each polymorphism showed that the men with AG alleles at the rs2842958 locus (*SOD2*) had lower levels (116.0 μg/L median) than those of men with GG alleles (121.8 μg/L median; $P = 0.03$, Table 3). This SNP (rs2842958) itself was not associated with the risk of aggressive disease.

Tests for interactions between plasma selenium level, gene variants and risk of aggressive prostate cancer are also reported in Table 3. Potential interactions of genotypes rs2758330 within *SOD2* and rs10432782 within *SOD1* with selenium and risk of aggressive prostate cancer were detected and explored further in Table 4. For both rs2758330 (*SOD2*) and rs10432782 (*SOD1*), the association of selenium levels with aggressive prostate cancer status was detected only at one genotype (Table 4). The RR for aggressive disease of men with GG or GT alleles (rs2758330) increased with increasing plasma selenium levels ($P_{trend} < 0.001$), with men in the highest quintile vs. lowest quintile having more than double the risk. However, selenium levels were not associated with the risk of aggressive disease among men who were T homozygous ($P_{interaction} = 0.02$ using quintiles of selenium, and 0.11 using a continuous measure of selenium). Similarly, for rs10432782, the RR for aggressive disease of men with T homozygote increased with their plasma selenium levels ($P_{trend} = 0.04$), while there was no significant association among men with GG or GT alleles ($P_{interaction} = 0.15$ or 0.05 using quintile or continuous measures of selenium, respectively; Table 4).

The results indicated that there were borderline associations between one *SOD1* haplotype and risk of aggressive prostate cancer, and that *SOD2* haplotypes modified the effect of selenium with disease aggressiveness. However, these associations were mainly driven by the single SNPs, as discussed above (data not shown). We gained no additional insights by a

haplotype analysis compared to results using single polymorphisms. Also there were no gene–gene interactions from either single polymorphism or haplotype analysis.

Discussion

In this study there were five SNPs within *SOD1* (rs10432782, rs17884057, rs4816407) and *SOD2* (rs2842958, rs2758330) that had suggestive associations with prostate cancer aggressiveness or selenium level, or that interacted with selenium level to affect the risk of prostate cancer aggressiveness. These data expand on previous work by ourselves [16,17] and others [27-30], that reported associations between a distinct variant in *SOD2* (rs4880) and prostate cancer risk or aggressiveness. *SOD1* and *SOD2* catalyse the same biochemical reaction, but have different characteristics in chromosomal location, cellular compartment, assembly of catalytic unit, and cofactors. Available information is very limited for these five SNPs, and to the best of our knowledge, this is the first report to describe the association of these *SOD2* and *SOD1* SNPs with prostate cancer status. However, the results should be interpreted cautiously, given the modest statistical power and likelihood for chance findings.

Two SNPs (rs17884057 and rs4816407) in *SOD1* were directly associated with the risk of presenting with aggressive prostate cancer; one variant in *SOD2* (rs2842958) correlated with selenium levels; and distinct variants in *SOD1* (rs10432782) and *SOD2* (rs2758330) had modifying effects on the associations between selenium and risk of aggressive prostate cancer. Selenium itself has been hypothesized to reduce cancer risk, including prostate cancer [31-52]. However, selenium supplementation did not affect the incidence of early-stage localized prostate cancer in the large randomized Selenium and Vitamin E Cancer Prevention Trial [53], and a few studies suggested that selenium might have an enhancing effect on cancer risk [31-33,43,54,55]. Selenium is involved in several enzymes of the ADS, forming an active centre of the enzymes GPX and thioredoxin reductase [18]. In cells, hydrogen peroxide (which is produced by the catalytic action of the SOD) is further detoxified to water by GPX, catalase, or peroxiredoxin; the activity of the last depending on a reduced form of thioredoxin, which is provided by thioredoxin reductase. In this manner, the SODs and selenium are indirectly co-operating in the ADS. However, to date no direct association between *SOD* and selenium has been shown at any level.

Genotype and/or external factors might be crucial in determining active levels of the SOD in cells and overall in the ADS. For example, the AA variant of rs4880 in *SOD2* might be more effective at transporting the enzyme through the mitochondrial membrane, thereby increasing breakdown of superoxide radicals into hydrogen peroxide [7]. Further breakdown of hydrogen peroxide into water relies on selenium-dependent GPX. If there is insufficient selenium, the GPX reaction is halted and an accumulation of hydrogen peroxide might occur, leading to toxicity, oxidation and propensity for DNA damage [16]. This *SOD2* variant (rs4880) has also been reported to modify associations of other risk factors for cancer: intake of fruits and vegetables [9] or smoking history [10] and breast cancer; age at diagnosis and colorectal cancer [11]; and race and lung cancer [14].

On *SOD1*, two polymorphisms (rs17884057, rs4816407) were associated with the risk of presenting with aggressive prostate cancer, and one (rs10432782) appeared to modify the effect of selenium on the risk of aggressive prostate cancer. LD analysis indicated that, among these three SNPs, none tagged the other two (threshold $r^2 \geq 0.8$ and LOD ≥ 3 [27]). Similarly, the LD analyses of variants studied in *SOD2* (rs2842958, rs2758330, and rs4880, previously reported) showed no tagging among these three SNPs (threshold $r^2 \geq 0.8$ and LOD ≥ 3, [27]).

Data on the association of *SOD* haplotype with diseases are limited. Wiener *et al*. [8] studied *SOD2* haplotypes and inherited Alzheimer's disease, and reported associations with four loci, rs2758346 (C or T), rs4880 (T or C), rs2855116 (T or G), and rs5747136 (G or A). In the current study, haplotype analysis showed no associations with aggressive prostate cancer beyond those indicated by single polymorphisms.

One recent study analysed more than 50 SNPs across 10 genes encoding proteins in the ADS (catalase, SOD1, SOD2, GPX1, GPX4, glutathione reductase, thioredoxin1 and 2, thioredoxin reductase 1 and 2 in relation to breast cancer [56]). In that study, two SNPs in *GPX4* (rs713041 and rs757229) were associated with all-cause mortality. In addition, there were some suggestions of antioxidant gene–gene interaction for breast cancer [57,58].

In conclusion, we identified several putative antioxidant-related genetic markers for the risk of aggressive prostate cancer. Further research is warranted to confirm or refute these results, in particular larger studies that expand target SNPs to other genes in the ADS, and consider metabolizing enzymes of exogenous antioxidants. Also, functional analyses for each potential SNP will be important.

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Abbreviations

ROS	reactive oxygen species
ADS	antioxidant defence system
SOD	superoxide dismutase
SNP	single-nucleotide polymorphism
GPX	glutathione peroxidase
PPARGC	peroxisome proliferators-activated receptor γ coactivator
XRCC	X-ray repair complementing defective repair in Chinese hamster cells
LD	linkage disequilibrium
LOD	logarithm of odds
RR	relative risk

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Table 1

The clinical and demographic characteristics of 753 men with localized prostate cancer at diagnosis, including 359 men with aggressive disease (e.g. intermediate/high prognostic risk)

Variable	Median (range; IQR) or n (%)
Age at diagnosis, years	62 (43–86; 56–68)
PSA at diagnosis, ng/mL	6.3 (0.7–575.8; 4.8–9.1)
Plasma selenium, μg/L (489 men)	121.4 (64.2–221.1; 110.4–135.1)
Ethnic group	
White	719 (95.5)
Other	30 (4)
Unknown	4 (0.5)
T stage at diagnosis	
T1b	1 (0.1)
T1c	392 (52.1)
T2	46 (6.1)
T2a	132 (17.5)
T2b	15 (2)
T3	5 (0.7)
T3a	3 (0.4)
T3b	1 (0.1)
Tx	158 (21)
Biopsy Gleason	
≤ 6	472 (62.7)
7	221 (29.3)
≤ 8	59 (7.8)
Unknown	1 (0.1)
PSA level at diagnosis, ng/mL	
≤ 10	589 (78.2)
10–20	104 (13.8)
> 20	55 (7.3)
Unknown	5 (0.7)
% of biopsy core positive	
≤ 33	331 (44)
33–50	100 (13.3)
> 50	168 (22.3)
Unknown	154 (20.5)
Risk categories*	
low	394 (52.3)
intermediate	259 (34.4)
high	100 (13.3)

*see text; aggressive prostate cancer defined as intermediate-/high-risk disease. IQR, interquartile range.

Table 2

SNPs studied among 753 men with localized prostate cancer at diagnosis

Gene	Gene ID	Chromosome	dbSNP ref no.	Allele	Function	Codon No.	Amino acid
GPX1	2876	3p21.3	–	$(GCG)_{4–6}$	repeat change	7~(10–12)*	(4–6)× Ala
			rs1050450	C/T	missense	198–200*	Pro/Leu
GPX4	2879	19p13.3	rs4807542	A/G	synonymous	12	Pro/Pro
			rs8178977	C/G	intronic		
			rs713041	C/T	synonymous	220	Leu/Leu
PPARGC1A	10891	4p15.1	rs8192678	A/G	missense	482	Ser/Gly
PPARGC1B	133522	5q33.1	rs17572019	A/G	missense	279	Ile/Val
			rs32577	C/T	synonymous	388	Pro/Pro
SOD1	6647	21q22.11	rs10432782	G/T	intronic		
			rs17884057	(–)/AGA	intronic		
			rs9967983	A/T	intronic		
			rs4816407	A/G	intronic		
SOD2	6648	6q25.3	rs2842958	A/G	intronic		
			rs4523113	A/T	intronic		
			rs2758330	G/T	intronic		
			rs5746136	A/G	3′-UTR	1264	
			rs5746138	A/G	3′-UTR	1496	
			rs7855	A/G	3′ near gene		
SOD3	6649	4q16.3-q21	rs8192287	G/T	5′ near gene		
			rs699473	C/T	5′ near gene		
			rs17878863	A/G	intronic		
			rs17881426	A/T	intronic		
			rs1007991	C/G	intronic		
			rs8192291	C/T	synonymous		
			rs2695232	C/T	3′-UTR	1011	
XRCC1	7515	19q13.3	rs25487	A/G	missense	399	Gln/Arg

*Codon number shifts, depending on (GCG) repeats. Polymorphisms captured by one of those listed: rs1800668 by rs1050450, rs7732671 by rs17572019, rs4998557 by rs10432782, rs2070424 and rs3216079 by rs4816407, rs2842960, rs2758339, rs5746112, rs2070994, rs4523113, rs2855116, rs2758332, rs2758331, & rs8031 by rs4880 [17], rs5746092, & rs5746094 by 4523113, rs8192288 by rs8192287, and rs2855262 by rs2695232.

Table 3

Genotype frequencies, selenium levels, and their associations with aggressive prostate cancer (intermediate- or high-risk disease) among 753 men with localized disease at diagnosis

		Genotype frequency (753)		Risk of aggressive cancer (753 t, 359 agg)		Selenium, μg/L (489 t, 359 agg)	P‡ for interaction (489)
Gene	Ref no	Genotype*	n (%)	n (%)	RR, P†	Median, P	
GPX1	GCGrepeats	4 4	125 (17)	60 (48)	1.00, 0.47	121.7, 0.33	0.83 (0.44)
GPX1	GCGrepeats	4 5	185 (25)	80 (43)	0.90 (0.70–1.15)	120.6	–
GPX1	GCGrepeats	4 6	167 (22)	85 (51)	1.06 (0.84–1.34)	122.4	–
GPX1	GCGrepeats	5 5	88 (12)	48 (55)	1.14 (0.87–1.48)	120.5	–
GPX1	GCGrepeats	5 6	132 (18)	58 (44)	0.92 (0.70–1.19)	118.5	–
GPX1	GCGrepeats	6 6	51 (7)	25 (49)	1.02 (0.73–1.43)	126.6	–
GPX1	rs1050450	C C	340 (46)	169 (50)	1.00, 0.10	122.6, 0.24	0.78 (0.67)
GPX1	rs1050450	C T	314 (42)	137 (44)	0.88 (0.74–1.04)	119.7	–
GPX1	rs1050450	T T	91 (12)	50 (55)	1.11 (0.89–1.37)	121.1	–
GPX4	rs4807542	G G	482 (68)	227 (47)	1.00, 0.78	121.8, 0.94	0.49 (0.31)
GPX4	rs4807542	A A/A G	224 (32)	108 (48)	1.02 (0.87–1.21)	121.4	–
GPX4	rs8178977	G G	442 (59)	207 (47)	1.00, 0.61	121.9, 0.67	0.08 (0.29)
GPX4	rs8178977	C C/C G	308 (41)	150 (49)	1.04 (0.89–1.21)	121.3	–
GPX4	rs713041	C C	211 (29)	97 (46)	1.00, 0.48	120.5, 0.66	0.49 (0.44)
GPX4	rs713041	C T	362 (49)	170 (47)	1.02 (0.85–1.23)	121.8	–
GPX4	rs713041	T T	166 (22)	86 (52)	1.13 (0.92–1.39)	123.1	–
PPARGC1A	rs8192678	G G	343 (46)	165 (48)	1.00, 0.67	121.3, 0.79	0.22 (1.00)
PPARGC1A	rs8192678	A G	322 (43)	148 (46)	0.96 (0.81–1.12)	121.2	–
PPARGC1A	rs8192678	A A	80 (11)	41 (51)	1.07 (0.84–1.35)	122.3	–
PPARGC1B	rs17572019	G G	634 (84)	302 (48)	1.00, 0.96	121.5, 0.70	0.21 (0.90)
PPARGC1B	rs17572019	A A/A G	117 (16)	56 (48)	1.00 (0.82–1.23)	121	–
PPARGC1B	rs32577	C C	544 (72)	254 (47)	1.00, 0.38	122, 0.16	0.59 (0.77)
PPARGC1B	rs32577	T/T T	209 (28)	105 (50)	1.08 (0.91–1.27)	118.7	–
SOD1	rs10432782	T T	600 (87)	283 (47)	1.00, 0.42	121.5, 0.86	0.14 (0.05)
SOD1	rs10432782	G G/G T	93 (13)	48 (52)	1.09 (0.88–1.36)	121.4	–
SOD1	rs17884057	AGA AGA	525 (70)	263 (50)	1.00, 0.04	121.7, 0.87	0.78 (0.67)

Gene	Ref no	Genotype frequency (753) Genotype*	n (%)	Risk of aggressive cancer (753 t, 359 agg) n (%)	RR, P†	Selenium, μg/L (489 t, 359 agg) Median, P	P‡ for interaction (489)
SOD1	rs17884057	(–)(–)/(–)AGA	223 (30)	93 (42)	0.83 (0.70–0.99)	120.7	–
SOD1	rs9967983	A A	262 (35)	124 (47)	1.00, 0.25	121.9, 0.90	0.81 (0.85)
SOD1	rs9967983	A T	332 (45)	150 (45)	0.95 (0.80–1.14)	121.2	–
SOD1	rs9967983	T T	150 (20)	80 (53)	1.13 (0.93–1.37)	121.4	–
SOD1	rs4816407	A A	644 (90)	299 (46)	1.00, 0.05	121.8, 0.07	0.58 (0.73)
SOD1	rs4816407	A G/G G	68 (10)	40 (59)	1.27 (1.02–1.57)	117.5	–
SOD2	rs2842958	G G	587 (85)	283 (48)	1.00, 0.97	121.8, 0.03	0.31 (0.55)
SOD2	rs2842958	A G	100 (15)	48 (48)	1.00 (0.80–1.24)	116	–
SOD2	rs4523113	T T	428 (58)	201 (47)	1.00, 0.63	121.2, 0.55	0.12 (0.08)
SOD2	rs4523113	A T/A A	314 (42)	153 (49)	1.04 (0.89,1.21)	121.8	–
SOD2	rs2758330	T T	482 (65)	234 (49)	1.00, 0.54	121.6, 0.46	0.02 (0.11)
SOD2	rs2758330	G G/G T	264 (35)	122 (46)	0.95 (0.81–1.12)	121.2	–
SOD2	rs5746136	G G	358 (50)	166 (46)	1.00, 0.53	121.2, 0.87	0.60 (0.49)
SOD2	rs5746136	A G	284 (40)	143 (50)	1.09 (0.92–1.27)	121	–
SOD2	rs5746136	A A	69 (10)	31 (45)	0.97 (0.73–1.29)	122.6	–
SOD2	rs5746138	A A	663 (88)	316 (48)	1.00, 0.99	121.8, 0.18	0.17 (0.25)
SOD2	rs5746138	A G/G G	88 (12)	42 (48)	1.00 (0.79–1.26)	119.2	–
SOD2	rs7855	A A	654 (90)	309 (47)	1.00, 0.92	121.5, 0.10	0.68 (0.94)
SOD2	rs7855	A G/G G	71 (10)	34 (48)	1.01 (0.78–1.31)	120.6	–
SOD3	rs8192287	G G	658 (88)	309 (47)	1.00, 0.26	121.4, 0.90	0.55 (0.19)
SOD3	rs8192287	G T/T T	92 (12)	49 (53)	1.13 (0.92–1.40)	121.5	–
SOD3	rs699473	T T	303 (41)	140 (46)	1.00, 0.78	121.2, 0.84	0.60 (0.40)
SOD3	rs699473	C T	328 (44)	160 (49)	1.06 (0.90–1.24)	121.6	–
SOD3	rs699473	C C	110 (15)	54 (49)	1.06 (0.85–1.33)	120.5	–
SOD3	rs17878863	A A	636 (85)	301 (47)	1.00, 0.79	121.1, 0.11	0.17 (0.74)
SOD3	rs17878863	A G/G G	113 (15)	55 (49)	1.03 (0.84–1.26)	124.4	–
SOD3	rs17881426	T T	602 (80)	287 (48)	1.00, 1.00	121.4, 0.70	0.71 (0.42)
SOD3	rs17881426	A A/A T	151 (20)	72 (48)	1.00 (0.83–1.21)	121.5	–
SOD3	rs1007991	G G	324 (45)	144 (44)	1.00, 0.45	121.4, 0.60	0.40 (0.29)
SOD3	rs1007991	C G	295 (41)	144 (49)	1.10 (0.93–1.30)	121.4	–

Gene	Ref no	Genotype frequency (753)		Risk of aggressive cancer (753 t, 359 agg)		Selenium, µg/L (489 t, 359 agg)	P‡ for interaction (489)
		Genotype*	n (%)	n (%)	RR, P†	Median, P	
SOD3	rs1007991	C C	98 (14)	49 (50)	1.12 (0.89–1.42)	120.2	–
SOD3	rs8192291	C C	470 (66)	214 (46)	1.00, 0.11	121.2, 0.26	0.48 (0.18)
SOD3	rs8192291	C T/T T	237 (34)	123 (52)	1.14 (0.97–1.33)	121.8	–
SOD3	rs2695232	T T	294 (40)	140 (48)	1.00, 0.89	121, 0.27	0.67 (0.15)
SOD3	rs2695232	C T	333 (45)	162 (49)	1.02 (0.87–1.20)	121.7	–
SOD3	rs2695232	C C	117 (16)	54 (46)	0.97 (0.77–1.22)	118.6	–
XRCC1	rs25487	G G	326 (43)	154 (47)	1.00, 0.76	121.4, 0.44	0.26 (0.32)
XRCC1	rs25487	A G	329 (44)	161 (49)	1.04 (0.88–1.21)	121	–
XRCC1	rs25487	A A	98 (13)	44 (45)	0.95 (0.74,1.22)	121.7, 0.33	0.83 (0.44)

*A rare homozygote was combined with a heterozygote if the frequency was < 0.05;

†estimated using the common homozygote as the reference group;

‡Test of interaction of genotypes and selenium levels on risk of aggressive prostate cancer. Selenium was evaluated as quintile groups as well as continuous values (in parentheses). t, total; agg, aggressive.

Table 4

The RR (95% CI) for aggressive prostate cancer according to quintiles of individual plasma selenium level and genotype status of individual polymorphisms in SOD1 and SOD2

Group	No. of patients	Quintile plasma selenium					p*	p†
		I (low)	II	III	IV	V (high)		
All patients	489	1.00 (ref)	0.99 (0.70–1.40)	1.08 (0.77–1.52)	1.11 (0.80–1.55)	1.35 (0.99–1.84)	0.04	
SOD1								
rs10432782								
T T	395	1.00 (ref)	1.02 (0.68–1.52)	1.25 (0.86–1.83)	1.20 (0.82–1.75)	1.42 (0.99–2.03)	0.04	0.15 (0.05)
G G/G T	60	1.00 (ref)	0.90 (0.47–1.72)	0.44 (0.19–1.02)	0.67 (0.29–1.53)	0.75 (0.38–1.50)	0.30	
SOD2								
rs2758330								
T T	311	1.00 (ref)	0.84 (0.58–1.22)	0.85 (0.58–1.25)	0.76 (0.50–1.15)	0.97 (0.68–1.37)	0.82	0.02 (0.11)
G G/G T	175	1.00 (ref)	1.23 (0.57–2.65)	1.71 (0.88–3.32)	2.10 (1.13–3.89)	2.58 (1.40–4.76)	< 0.001	

*Trend;

†interaction of genotypes and selenium levels on risk of aggressive prostate cancer. Selenium was evaluated as quintile groups as well as continuous values (in parentheses).