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Plasma Selenium, Manganese Superoxide Dismutase, and Intermediate- or High-Risk Prostate Cancer

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ABSTRACT

Purpose

In vitro, in vivo, and epidemiologic studies support a role for selenium in reducing the risk of prostate cancer. Our group previously demonstrated a strong interaction between plasma selenium and the manganese superoxide dismutase (*SOD2*) gene and incident prostate cancer risk. We now hypothesized that *SOD2* modifies the association between selenium level and risk of aggressive prostate cancer at diagnosis.

Patients and Methods

We assessed *SOD2* variants and plasma selenium in 489 patients with localized/locally advanced prostate cancer from an ongoing retrospective cohort. Cross-sectional associations with aggressive prostate cancer (ie, stage T2b-3, prostate-specific antigen > 10 ng/mL, or biopsy Gleason score $\geq$ 7) were evaluated using the χ^2 test, Cochran-Armitage test for trend, and estimations of relative risk (RR) and 95% CIs.

Results

SOD2 genotype alone was not associated with disease aggressiveness, whereas higher versus lower selenium levels were associated with a slightly increased likelihood of presenting with aggressive disease (RR = 1.35; 95% CI, 0.99 to 1.84). There was evidence of an interaction between *SOD2* and selenium levels such that among men with the AA genotype, higher selenium levels were associated with a reduced risk of presenting with aggressive disease (RR = 0.60; 95% CI, 0.32 to 1.12), whereas among men with a V allele, higher selenium levels were associated with an increased risk of aggressive disease (for VV or VA men, RR = 1.82; 95% CI, 1.27 to 2.61; *P* for interaction = .007).

Conclusion

These data suggest that the relationship between circulating selenium levels at diagnosis and prognostic risk of prostate cancer is modified by *SOD2* genotype and indicate caution against broad use of selenium supplementation for men with prostate cancer.

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INTRODUCTION

Experimental and epidemiologic studies indicate that selenium may reduce prostate cancer risk, potentially through inhibition of cellular proliferation, apoptosis, antiangiogenesis, or antioxidant pathways.¹⁻²² A protective effect of higher versus lower endogenous selenium levels on incident prostate cancer risk has been reported in large observational prospective studies.^{1,2,4,5,7-10,14,17} In the Nutritional Prevention of Cancer Trial, secondary data analyses revealed a 50% reduction in incident prostate cancer risk with daily supplementation of 200 μ g of selenium (mean follow-up time = 7.4 years).^{6,23} A few studies have reported that the inverse association for endogenous selenium and prostate cancer risk was more pronounced among

smokers, multivitamin users, and men with high vitamin E intake or circulating level^{1,8,17} or who had higher diagnostic stage or prostate-specific antigen (PSA) level.^{9,14}

In contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) recently ended early (5.5 years of median follow-up) based on convincing interim analyses that indicated that neither selenium nor vitamin E, alone or in combination, was effective (at the tested doses and formulations) for the primary prevention of prostate cancer.^{24,25} Although this trial provided strong evidence for a null association for oral supplementation with L-selenomethionine 200 μ g/d and overall prostate cancer risk, it did not address the questions of whether higher selenium levels affect the risk of incident advanced-stage prostate cancer

or risk of progression in men who already have prostate cancer and how genetics may modify associations between selenium and prostate cancer. On the basis of this current report and our previous reports,²⁶ it is possible that selenium intervention for prostate cancer may still be important for men of specific genotypes or with specific tumor phenotypes, and further understanding of gene-diet interactions may help elucidate findings from SELECT.

Our collaborative group previously demonstrated a strong interaction between plasma antioxidants and a gene variant in manganese superoxide dismutase (*SOD2*) and incident prostate cancer risk.²⁶ *SOD2* is an endogenous mitochondrial enzyme that metabolizes reactive oxygen species and superoxide anions to oxygen and hydrogen peroxide. The latter is further converted to water via catalase, glutathione peroxidase (GPX), or peroxiredoxin, and GPX and peroxiredoxin are selenium dependent. In a nested case-control study within the Physicians' Health Study (PHS), our group reported a strong interaction between the valine (V)/alanine (A) polymorphism (rs4880) in *SOD2* and plasma selenium and risk of total and aggressive prostate cancer.²⁶ Men who were in the highest quartile of selenium and had the AA genotype had more than a 50% reduction in risk of total (odds ratio = 0.47; 95% CI, 0.26 to 0.85; *P* for interaction = .05) and aggressive (odds ratio = 0.35; 95% CI, 0.15 to 0.82; *P* for interaction = .01) prostate cancer compared with men who were in the lowest quartile of selenium and had a V allele. Among V allele carriers, the effect of high versus low selenium level on prostate cancer risk was in the protective direction but weaker and not statistically significant. Although the exact biologic mechanism of the variant is unknown, the valine variant of *SOD2* may result in less efficient transport of *SOD2* into the mitochondrial matrix, which compromises the cell's ability to neutralize superoxide radicals.²⁷ To expand on this work, we examined a distinct cohort of 489 men with prostate cancer and hypothesized that *SOD2* would modify the association between plasma selenium level and risk of presenting with aggressive prostate cancer at diagnosis.

PATIENTS AND METHODS

Study Population

This work was conducted within an ongoing cohort comprised of patients with localized/locally advanced prostate cancer (ie, stage T3 or less, N0, and M0) with banked biospecimens at the Dana-Farber Cancer Institute, Boston, MA. Patients had to have consented and donated blood for research before undergoing any type of local therapy and consented to clinical research follow-up.²⁸ Of 778 patients who fulfilled these criteria, 764 had sufficient DNA available, and 499 had sufficient plasma for selenium assessment. Four hundred eighty-nine had complete clinical, selenium, and genotype data and were included in this analysis. These men were diagnosed with prostate cancer between January 1994 and March 2001. Median time from diagnosis to blood draw was 44 days (interquartile range, 31 to 60 days), and four patients had blood drawn more than 180 days after diagnosis.

Genomic DNA

Genomic DNA was prepared from peripheral blood using QIAamp DNA Blood mini kit (Qiagen Inc, Valencia, CA). DNA concentration was assayed using PicoGreen (Invitrogen, Carlsbad, CA) and adjusted to 5 ng/ μ L for genotyping.

Genotyping

Genotyping of rs4880 (C or T) on *SOD2* was performed by Increased Plexing Efficiency and Flexibility for MassARRAY System Assay (Sequenom, San Diego, CA) through single base primer extension with mass-modified

terminators. This polymorphism locates on exon 2, at nucleotide position 47 (counting from the initial methionine codon).²⁹ C(GCT) type encodes alanine (A), and T(GTT) encodes valine (V). This polymorphism is shown as Ala16Val in a precursor form and Ala-9Val in a mature form.³⁰ Six percent of samples were analyzed twice with blind numbering as internal quality control samples, and there was complete concordance. Hereafter, we refer to these gene variants as AA, VA, and VV.

Plasma Selenium Assessment

Selenium assay methods of this lab (I.B.K.) have been published previously.³¹ Briefly, selenium concentration was assessed by flameless atomic

Table 1. Patient Demographics and Clinical Characteristics at Diagnosis of Men With Prostate Cancer (N = 489)

Characteristic	No. of Patients	%
Age at diagnosis, years		
Median	62	
Range	43-86	
Interquartile range	57-68	
PSA at diagnosis, ng/mL		
Median	6.0	
Range	0.7-575.8	
Interquartile range	4.7-8.3	
Plasma selenium, μ g/L		
Median	121.4	
Range	64.2-221.1	
Interquartile range	110.4-135.1	
Race		
White	468	95.7
Other	18	3.6
Unknown	3	0.6
T stage at diagnosis		
T1c	259	53
T2	21	4.3
T2a	94	19.2
T2b	9	1.8
T3	2	0.4
T3a	1	0.2
Tx	103	21.1
Biopsy Gleason sum		
6 or less	312	63.8
7*	153	31.3
8 or more	24	4.9
PSA at diagnosis, ng/mL		
≤ 10	405	82.8
10-20	52	10.6
> 20	27	5.5
Unknown	5	1.0
% of biopsy core positive		
≤ 33	230	47
33-50	59	12.1
> 50	109	22.3
Unknown	91	18.6
Disease risk categories†		
High	46	9.4
Intermediate	167	34.2
Low	276	56.4

Abbreviation: PSA, prostate-specific antigen.

*Gleason sum 3 + 4, n = 94; Gleason sum 4 + 3, n = 45; and Gleason sum components unknown, n = 14.

†D'Amico risk categories are as follows: low risk ($\leq$ T2a, PSA ≤ 10 ng/mL, and Gleason sum ≤ 6), intermediate risk (T2b, PSA = 10-20 ng/mL, or Gleason sum = 7), and high risk (> T2b, PSA > 20 ng/mL, or Gleason sum > 7).

absorption (Perkin-Elmer 800; Perkin-Elmer, Norwalk, CT) using an electrodeless discharge lamp operating at $\lambda = 196.0$ nm and I'Vov platform graphite furnace (Perkin-Elmer) with Zeeman background correction.³² Samples were run bracketed by quality control samples (ie, certified standard reference material no. 66816 from Utak Laboratory, Valencia, CA) with criteria for acceptance coefficient of variation less than 10%; 5% of study samples were analyzed in blinded duplicates (coefficient of variation = 5.3%). This research was approved by the institutional review boards of all collaborating institutions.

Statistical Analysis

Patient disease characteristics at diagnosis and *SOD2* genotype (rs4880) frequencies were summarized as the number and percentage of patients or as the median, range, and interquartile range of levels. Plasma selenium levels were categorized according to quintile cutoff values (108.3, 118.0, 125.5, and 139.8 $\mu\text{g/L}$, equivalent to 1.08, 1.18, 1.26, and 1.40 ppm, respectively).

The primary outcome of interest was presentation of aggressive prostate cancer at diagnosis. Aggressive disease was defined as clinical stage T2b-3, PSA more than 10 ng/mL, or biopsy Gleason sum ≥ 7 (corresponding to D'Amico intermediate-/high-risk categories³³). Although central pathology review was not conducted solely for the current study, 94% of participants had their slides reviewed by a single pathology group, Brigham and Women's Hospital Department of Pathology (Boston, MA). Associations of disease aggressiveness with *SOD2* and selenium levels were evaluated using the χ^2 test or the Cochran-Armitage test for trend. Relative risk (RR) and 95% CIs were estimated using a generalized linear model for binomial data with a log link rather than a logit link function. We also conducted similar analyses using logistic regression models to compute prevalence odds ratios as estimates of the RR. Results were similar, and we present here the results of the log-binomial models only because this method may be superior with regard to providing estimates of 95% CIs from cross-sectional data.³⁴ The likelihood ratio test from the generalized linear model was used to test for an interaction between *SOD2* and selenium on disease aggressiveness. There was insufficient variation in this population to examine effects separately by race. All analyses were conducted using SAS version 9 (SAS Institute, Cary, NC), and $P < .05$ (two-sided) was considered statistically significant.

RESULTS

Demographic and clinical characteristics of this cohort of prostate cancer survivors are listed in Table 1. Median age was 62 years, and median PSA level was 6.0 ng/mL in this predominantly white population. More than half the cohort had good risk disease, whereas approximately one third was intermediate risk, in part reflecting the original cohort entry criteria. Neither age ($P = .38$) nor race ($P = .69$) was associated with risk of presenting with aggressive disease.

Median selenium level was similar among men with different *SOD2* genotypes (approximately 121 $\mu\text{g/L}$), and *SOD2* alone was not associated with risk of presenting with aggressive prostate cancer (Table 2). Higher plasma selenium level was slightly associated with in-

creased risk of having aggressive prostate cancer at diagnosis (Table 3). This effect was statistically significantly modified by *SOD2* genotype. Among men with the AA genotype, those in the highest versus lowest quintile of selenium had a borderline statistically significant 40% lower risk of aggressive prostate cancer (P for trend = .06). In contrast, among men with a V allele, those with high versus low plasma selenium had an increased risk of presenting with aggressive prostate cancer (RR = 2.48; 95% CI, 1.07 to 5.71; P for trend = .04 for VV; and RR = 1.59; 95% CI, 1.07 to 2.35; P for trend = .004 for VA; Fig 1 and Table 3). Overall, there was a statistically significant interaction between *SOD2* and selenium and risk of aggressive prostate cancer ($P = .007$; Table 3 and Fig 1). This interaction remained evident in a cross-stratified analysis of all patients using a single reference group (ie, lowest quintile of selenium and having a V allele; Table 3). In this model, the association between selenium level and aggressive disease was clearly dependent on *SOD2*, with those in the highest versus lowest quintile of selenium having either no association or almost a two-fold increase in risk, depending on *SOD2* variant (Table 3).

Results reflected in Table 3 were similar when age and race were included in the multivariate models (P for interaction = .02), when we adjusted for year of diagnosis (P for interaction = .004), when we examined clinical Gleason sum as a single outcome variable (Gleason ≥ 7 ; P for interaction = .04); and when we used logistic regression models to compute prevalence odds ratios as estimates of the relative risk (P for interaction = .007; estimates consistent and stronger; data not shown).

DISCUSSION

We observed strong support for the hypothesis that plasma selenium levels and *SOD2* genotype interact to influence risk of presenting with aggressive prostate cancer at diagnosis in men with localized or locally advanced prostate cancer. Higher selenium levels alone were also slightly positively associated with worse prognostic risk at diagnosis, which largely reflected an association with higher Gleason sum. There was no association for *SOD2* alone and prostate cancer aggressiveness in this population.

The median selenium level in this population (121.4 ng/mL) was comparable to that in several other studies, where the range of median (or mean) circulating selenium levels among healthy control or placebo groups was 108 to 141 ng/mL.^{1,5,7,8,14,23,25} The trend toward a slightly higher risk of aggressive disease for higher levels of selenium in this case population is unique and somewhat in contrast to several

Table 2. Distribution of *SOD2* Genotypes and Association With Aggressive Disease and Plasma Selenium Levels Among 489 Men With Prostate Cancer

Genotype	Patients		Aggressive Disease			Selenium Level ($\mu\text{g/L}$)		
	No.	%	Relative Risk	95% CI	P^*	Median	Range	$P^\dagger$
VV	107	21.9	1.00	Reference	.346	120.9	85.4-221.1	.376
VA	252	51.5	1.21	0.92 to 1.59		121.5	64.2-211.6	
AA	130	26.6	1.10	0.81 to 1.51		121.7	84.2-193.5	

* χ^2 test.

†Kruskal-Wallis test.

Table 3. RR for Aggressive Prostate Cancer According to Quintiles of Plasma Selenium for Men With Prostate Cancer and Stratified by *SOD2* Genotype

Genotype	No. of Patients	Quintile Levels of Plasma Selenium										P* (trend)	P† (interaction)
		I (low)		II		III		IV		V (high)			
		RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI		
All patients	489	1.00		0.99	0.70 to 1.40	1.08	0.77 to 1.52	1.11	0.80 to 1.55	1.35	0.99 to 1.84	.042	
SOD2 genotype grouped separately													
VV	107	1.00		1.58	0.64 to 3.85	1.95	0.83 to 4.58	1.73	0.74 to 4.06	2.48	1.07 to 5.71	.043	.046
VA	252	1.00		0.80	0.47 to 1.35	1.09	0.70 to 1.71	1.20	0.77 to 1.87	1.59	1.07 to 2.35	.004	
AA	130	1.00		0.95	0.57 to 1.59	0.65	0.32 to 1.30	0.71	0.39 to 1.31	0.60	0.32 to 1.12	.056	
SOD2 genotype with VV and VA groups combined													
VV/VA	359	1.00		0.98	0.62 to 1.53	1.29	0.87 to 1.92	1.32	0.89 to 1.97	1.82	1.27 to 2.61	.0003	.007
AA	130	1.00		0.95	0.57 to 1.59	0.65	0.32 to 1.30	0.71	0.39 to 1.31	0.60	0.32 to 1.12	.056	
SOD2 genotype with VV and VA groups combined and using a common reference													
VV/VA	359	1.00		0.98	0.62 to 1.53	1.29	0.87 to 1.92	1.32	0.89 to 1.97	1.82	1.27 to 2.61	—	.007
AA	130	1.56	0.97 to 2.53	1.49	0.93 to 2.39	1.01	0.52 to 1.98	1.11	0.62 to 1.98	0.93	0.51 to 1.69		

Abbreviations: RR, relative risk.

*Cochran-Armitage test for trend.

†From the generalized linear model where selenium was coded as quintile levels.

studies that reported decreased prostate cancer risk with higher selenium levels.^{1,2,4,5,7-10,14,17} However, some data indicate that the association between selenium and cancer risk may not be entirely linear and that higher doses could have null or adverse effects.

In SELECT, men receiving selenium supplementation had a median selenium level that was substantially higher (252 $\mu\text{g/L}$) than that of men in the current study (121 $\mu\text{g/L}$; range, 64 to 221 $\mu\text{g/L}$), and the trial reported null results for selenium supplementation versus placebo and incident (mainly early-stage) prostate cancer risk and a nonsignificant elevation in diabetes risk.²⁵ In the large National Institutes of Health–American Association of Retired Persons Diet and Health Study, more than daily usage of multivitamins was associated with an elevated risk of incident advanced and fatal prostate cancer, in particular among men who also took individual supplements of selenium, β -carotene, or zinc or who had a family history of prostate cancer.³⁵ Waters et al¹⁹ conducted a randomized controlled feeding study of selenium supplementation versus placebo in male dogs, the only other species besides humans to experience spontaneous prostate cancer. Dogs receiving selenium had significantly less DNA damage and more apoptosis in their peripheral-blood lymphocytes and prostate cells,¹⁹ and there was a U-shaped dose-response curve between toenail measures of selenium and extent of DNA damage in prostate tissue.³⁶ In the Nutritional Prevention of Cancer Trial, selenium supplementation reduced total and prostate cancer incidence; however, this effect was limited to those with lower baseline circulating selenium levels (hazard ratio = 0.14; 95% CI, 0.03 to 0.61 for selenium levels < 106.4 ng/mL). Among men in the highest tertile (> 123.2 ng/mL), selenium supplementation was unassociated with prostate cancer risk, although in the positive direction (hazard ratio = 1.14; 95% CI, 0.51 to 2.59).¹³ Also, among those randomly assigned to selenium, being in the highest versus lowest tertile of circulating selenium at baseline was associated with an 88% increased risk of total cancer ($P = .01$).²³ Together, these data suggest that obtaining a certain level of selenium may confer some protection against prostate cancer; however, the

relationship may not be linear, and caution is warranted before assuming that obtaining increasingly higher levels confers increased benefit.

The effect of selenium status on prostate cancer may also depend on how it interacts with components of the oxidative stress pathway, as evidenced by the current study. The observed 40% decreased risk of aggressive prostate cancer associated with higher selenium among men with the AA variant (P for trend = .06) was consistent and expands on our previous findings in the PHS. However, an important difference was that in the current study, men with a V allele had an elevated risk of aggressive disease with higher selenium levels, and there was no such trend in the PHS. Differences in study design and patient population may explain this apparent inconsistency. In particular, PHS focused on prediagnostic selenium levels from years before diagnosis, in contrast to the present study, which examined levels at or shortly after diagnosis. Also, we contrasted cases only, whereas the PHS compared men with and without prostate cancer diagnoses to examine risk of developing total or advanced prostate cancer. Furthermore, in the PHS, the significant inverse relation was observed only for risk of developing advanced-stage, not localized, prostate cancer; and in the current study, we examined only patients that had stage T3 disease or less. Despite these differences, the findings converge on demonstrating an interaction of selenium with *SOD2* genotype in prostate cancer.

Several other studies have reported on possible effect modification by *SOD2* on the relationship between dietary factors and prostate cancer incidence.³⁷⁻⁴⁰ In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, having *SOD2* AA genotype was associated with an increased risk of developing prostate cancer, in particular among those with lower vitamin E intake who were also smokers, although there was no effect modification by selenium level.³⁸ In the Carotene and Retinol Efficacy Trial, *SOD2* alone was not associated with prostate cancer risk, but among VV carriers, never versus ever use of vitamin supplements or greater iron intake was associated with double the risk of incident prostate cancer.^{37,40} In contrast, *SOD2* AA

Fig 1. Relative risk and 95% CI for aggressive prostate cancer according to quintiles of plasma selenium level (A) by each *SOD2* genotype and (B) for AA and the combined group of VV or VA *SOD2* genotype.

genotype (ν VV or VA) was associated with more than 2.5-fold elevation in risk of high-grade prostate tumors in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, whereas there was no evidence for an interaction by vitamin E or β -carotene.³⁹ A few other studies have also reported a higher frequency of A alleles among prostate cancer patients versus controls or healthy adults.^{41,42} Further evidence that *SOD2* genotype modifies diet or lifestyle associations with carcinogenesis comes from studies of breast,⁴³⁻⁴⁸ bladder,⁴⁹ and skin⁵⁰ cancer.

The observed interaction between *SOD2* and selenium levels may reflect the dual roles of selenium as a pro- and antioxidant, depending on dose, form, and function of other enzymes.^{51,52} The AA variant of *SOD2* may be more effective at transporting the enzyme through the mitochondrial membrane, thereby increasing breakdown of superoxide radicals into hydrogen peroxide.³⁰ If there is sufficient selenium, then (selenium-dependent) GPX can next act to efficiently break down hydrogen peroxide into water. However, if there is not sufficient selenium, the GPX reaction is halted, and a buildup of hydrogen

peroxide may occur, leading to toxicity, oxidation, propensity for DNA damage, and increased cancer risk. This may be one way in which lower versus higher selenium levels and the AA variant could be associated with an increased risk of aggressive prostate cancer.²⁶

The direct relation of higher selenium with more aggressive disease among carriers of the V allele was unexpected and has not previously been reported. One explanation might be that in men with established cancer, antioxidants may promote cancer cell survival through an antiapoptotic mechanism by neutralizing the higher levels of reactive oxygen species found in cancer cells. Alternatively, given the cross-sectional nature of our study, it could be that prostate tumors that develop despite high selenium status tend to have higher Gleason grade. However, the interaction and different associations observed based on *SOD2* argues against this reverse causality explanation. One could speculate that if there is chronic lower normal *SOD2* activity and high superoxide anion production as a result of high concentrations of selenite, then there may be an imbalance toward oxidative stress that cannot be fully compensated by GPX or catalase actions alone.⁵¹ Finally, this finding may be a result of chance and warrants further confirmation in humans.

This study has several limitations that must be considered. It was cross-sectional, and we cannot entirely rule out the possibility that the slight positive association for selenium and risk of aggressive disease was a result of patients with worse clinical features taking selenium supplements out of concern about their diagnosis. However, most blood was donated shortly after diagnosis, and the strong interaction with *SOD2* genotype argues against this. Other limitations include our inability to adjust for potential confounding factors, such as body size or diet, and lack of racial diversity.

In conclusion, this cross-sectional analysis of 489 men with prostate cancer provides evidence that selenium levels interact with *SOD2* genotype to influence the risk of presenting with aggressive prostate cancer at diagnosis. Among the approximate 25% of men with the AA genotype, having greater selenium levels may protect against aggressive disease. However, for the 75% men who carry a V allele, higher selenium levels might increase the likelihood of having worse tumor characteristics. Together, these data underscore the importance of genotype for understanding the potential benefits and dangers of selenium on prostate cancer and may, in part, explain apparently conflicting results from previous studies. Complete interpretation of results from SELECT may depend on assessment of *SOD2* genotype in trial participants.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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