Manganese Superoxide Dismutase Polymorphism, Prediagnostic Antioxidant Status, and Risk of Clinical Significant Prostate Cancer

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Abstract

Oxidative stress may enhance prostatic carcinogenesis. A polymorphism [valine (V) → alanine (A)] of manganese superoxide dismutase (MnSOD), the primary antioxidant enzyme in mitochondria, has been recently associated with prostate cancer. We examined the relationship between prostate cancer and the MnSOD polymorphism and its interactions with baseline plasma antioxidant levels (selenium, lycopene, and α-tocopherol) and β-carotene treatment among 567 cases and 764 controls nested in the prospective Physicians' Health Study. We found little overall association between MnSOD polymorphism and prostate cancer risk; however, this polymorphism significantly modified risk of prostate cancer associated with prediagnostic plasma antioxidants ($P_{interaction} \leq 0.05$). Among men with the AA genotype, high selenium level (4th versus 1st quartile) was associated with a relative risk (RR) of 0.3 [95% confidence interval (CI), 0.2-0.7] for total prostate cancer; for clinically aggressive prostate cancer, the RR was 0.2 (95% CI, 0.1-0.5). In contrast, among men with the VV/VA genotype, the RRs were 0.6 (0.4-1.0) and 0.7 (0.4-1.2) for total and clinically aggressive prostate cancer. These patterns were similar for lycopene and α-tocopherol and were particularly strong when these antioxidants and selenium were combined; men with the AA genotype had a 10-fold gradient in risk for aggressive prostate cancer across quartiles of antioxidant status. Men with AA genotype who were randomly assigned to β-carotene treatment (versus placebo) had a RR of 0.6 (95% CI, 0.2-0.9; $P_{interaction} = 0.03$) for fatal prostate cancer, but no significant association was observed in men with the VV/VA genotype. Both endogenous and exogenous antioxidants play an important and interdependent role in preventing clinically significant prostate cancer. (Cancer Res 2005; 65(6): 2498-504)

Introduction

Oxidative damage caused by reactive oxygen species and other free radicals is involved in prostatic carcinogenesis (1). Manganese superoxide dismutase (MnSOD) is the primary antioxidant in the mitochondria. This enzyme converts reactive oxygen species to oxygen and hydrogen peroxide, and the latter is catalyzed into water by catalase and glutathione peroxidase, a selenium enzyme. Laboratory evidence indicates that MnSOD functions as a tumor suppressor, possibly by modulating apoptotic and proliferation pathways *in situ* (2, 3).

A polymorphism encoding for either valine (V) or alanine (A) at codon 16 in the mitochondrial targeting sequence of the human *MnSOD* gene was previously described (4). The allele frequency for A is 12% among Japanese (4), whereas it is more common (41-55%) in the Caucasian population (5). Although the function of this polymorphism requires further elucidation, it is suggested that this polymorphism alters the secondary structure of the protein, and hence may affect the efficiency of mitochondrial transport of MnSOD (4). Sutton et al. (6) recently showed that A-MnSOD allows more efficient MnSOD import into the mitochondrial matrix and generates more active MnSOD compared with the V-variant, suggesting that AA homozygous subjects may have higher MnSOD activity than VV subjects.

According to these observations, one might predict that having the AA genotype is beneficial; however, several studies have suggested increased cancer risk associated with the AA genotype, particularly among individuals with heavy oxidative stress or with poor dietary antioxidant status. In a cohort of Finnish male heavy smokers (7), men homozygous for the A allele had a 70% increase in total prostate cancer risk and a 3-fold increase in risk of high-grade tumors (Gleason score of 8-10) compared with those with the MnSOD VV or VA genotype. Five studies examined the influence of this polymorphism on breast cancer incidence (5, 8–11) and two indicated a moderate elevation (1.5-4.3-fold) in risk among women homozygous for the A allele (5, 8). In particular, Ambrosone et al. (5) found that breast cancer risk was elevated among premenopausal women with the AA genotype who had low consumption of dietary antioxidants; similar findings were reported in a study by Cai et al. (11) in Shanghai, China. Tamimi et al. (10) observed an overall null association with breast cancer, but among current heavy smokers, women with the AA (versus VV) genotype had a >4-fold increased risk [odds ratio (OR), 4.53; 95% confidence interval (CI), 0.89-23.16]. Studies of other cancer sites have been inconsistent (12–14) and no study has evaluated the interaction between this polymorphism and plasma antioxidant status.

Therefore, we conducted this nested case-control study within the Physicians' Health Study to investigate the association between MnSOD polymorphism and risk of prostate cancer. We previously found lower risks of total or high-stage (C and D) prostate cancer

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among men with higher baseline dietary or biomarker levels of the antioxidants, selenium (15, 16), lycopene (17, 18), or vitamin E (17, 19). Also, a randomized, double-blind clinical trial showed that selenium supplementation significantly lowered the incidence of prostate cancer (20, 21). Given the key role of selenium as a component of glutathione peroxidase in the MnSOD antioxidative pathway, we specifically evaluated the interaction between the MnSOD polymorphism and status of the exogenous dietary antioxidants—selenium, lycopene, α-tocopherol, and γ-tocopherol—in modifying prostate cancer carcinogenesis. We also assessed whether the MnSOD polymorphism modifies the relationship between β-carotene assignment and prostate cancer risk in the randomized Physicians' Health Study trial.

Materials and Methods

Study Population. The Physicians' Health Study was a randomized, double-blind, placebo-controlled trial of 325 mg aspirin and 50 mg β-carotene, every other day, among 22,071 healthy U.S. male physicians, ages 40 to 84 years, that began in 1982. Men were excluded at baseline if they had a history of myocardial infarction, stroke, transient ischemic attack, or unstable angina; cancer (except for nonmelanoma skin cancer); current renal or liver disease, peptic ulcer, gout; or current use of platelet-active agents, vitamin A, or β-carotene supplements. Men provided medical information via mailed-in questionnaires, and 14,916 (68%) provided blood before randomization (22, 23). The participants were predominately Caucasian (93%). Through 1995, follow-up was over 99% complete; vital status was ascertained for 100% by 2002. The aspirin arm was terminated at the end of the fifth year due to a reduction in the risk of myocardial infarction, and β-carotene component of the trial continued until 1995.

Study physicians, unaware of the questionnaire or assay data, verified the reports of prostate cancer by participants and reviewed medical records and pathological reports to determine the tumor Gleason score, grade, and stage according to the modified Whitmore-Jewett classification scheme (24). Cases without pathologic staging were classified as indeterminate stage unless there was clinical evidence of distant metastases.

Prostate cancer cases for the current study were drawn from participants who provided blood specimens at baseline. For each case, we selected one to two controls at random from those who had provided blood, had not had a prostatectomy, and had not reported a diagnosis of prostate cancer at the time the diagnosis was reported by the case subject. Controls were individually matched to cases by age (±1 year for men ages ≤55 years and ±5 years for men ages >55 years) and smoking status (never, former, or current).

Laboratory Assessment. MnSOD (V → A) polymorphism was successfully genotyped for 567 cases and 764 controls, representing 92% of the eligible subjects. Genomic DNA (40 ng) was extracted from peripheral blood and was amplified using 17 pmol primers (5′-GTAGCACCAGCACTAGCAGCAT-3′ and 5′-GCGTTGATGTGAGGTTCCAG-3′) in PCR buffer (Qiagen, Chatsworth, CA). PCR had an initial denaturing temperature of 94°C (1 minute) followed by 30 cycles of denaturing (94°C, 1 minute), annealing (61°C, 30 seconds), and extension (72°C, 45 seconds). A 7-minute extension at 72°C followed the final cycle. PCR product was digested with *Bsa*WI (60°C, 1 hour; New England BioLabs, Beverly, MA). Digested products were visualized on a 2% agarose gel stained with ethidium bromide. Fragment patterns specific for three MnSOD genotypes were VV (GTT; 351 bp, 87 bp), VA (GTT/GCT; 438 bp, 351 bp, 87 bp), and AA (GCT; 438 bp). The laboratory personnel were unaware of case-control status. To assess genotyping reproducibility, they repeated a random 10% selection of the samples; all genotypes matched initial designated genotypes.

Plasma levels of carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene), vitamin E (α- and γ-tocopherol), retinol, and selenium were measured as previously described (16, 17). Mean intra-assay coefficients of variation for these antioxidants ranged from 6.4% for selenium to 11.9% for α-tocopherol.

Statistical Analysis. To examine the association of the MnSOD polymorphism with prostate cancer risk, we compared allele and genotype frequencies between cases and controls using the χ^2 test. We subsequently assessed the association for total prostate cancer and refitted models for nonaggressive and aggressive prostate cancer, respectively. Men diagnosed with stage C or D, high-grade prostate cancer (Gleason score of 7-10), and those who died from prostate cancer during the follow-up were categorized as having aggressive disease; among the rest, those diagnosed with stage A or B and low-grade prostate cancer (Gleason score of 2-6) were categorized as having nonaggressive disease. Cases (n = 11) with unknown disease status were excluded from the subgroup analyses.

We evaluated the potential interactions between MnSOD polymorphism and baseline plasma antioxidant levels, including selenium, vitamin E (α- and γ-tocopherol), carotenoids (lycopene, α- and β-carotene, β-cryptoxanthin, lutein), and retinol in relation to prostate cancer risk. In addition, we investigated the interaction of MnSOD polymorphism with randomization to either β-carotene or placebo at baseline. Because of meaningful patterns observed from the interaction models for selenium, α-tocopherol and lycopene and our previous findings of significant inverse associations between each of these antioxidants and prostate cancer risk independently in two cohorts (15–19); as a secondary analysis, we derived an antioxidant score to assess the association of combined plasma antioxidant status with prostate cancer risk.

For all analyses, we first examined the association for MnSOD genotypes VA and AA, using VV as the reference. Because the results for the VV and VA genotypes were similar, the two were collapsed into a single reference group. Relative risks (RR) and 95% CI were calculated for total, nonaggressive, and aggressive prostate cancer using unconditional logistic regression; we included all controls in subgroup analyses stratified by disease status to maximize statistical power. All models were adjusted for age at baseline, smoking status, and duration of follow-up, the matching factors for control selection. The duration of follow-up was calculated in years between baseline and diagnosis for cases and the duration for a control was the same as the matched case.

We assigned each study participant to a quartile according to cutpoints (batch-specific cutpoints for carotenoids, vitamin E, and retinol) from the control subjects. An antioxidant score (range 3-12, low to high) was derived for each man according to the sum of his quartile levels of selenium, α-tocopherol, and lycopene. Based on this derived score, we categorized all men into four groups with antioxidant scores of 3 to 5, 6 to 7, 8 to 9, and 10 to 12. To test the interaction between MnSOD polymorphism and prediagnostic antioxidant status, we tested models with and without the product term of the MnSOD genotype with the quartile median level of an antioxidant (for plasma levels of individual antioxidants), with the antioxidant score (for overall plasma levels of antioxidants), or β-carotene treatment (yes or no).

Overall, we had 567 cases and 764 controls to examine the association between MnSOD polymorphism and risk of prostate cancer and the interaction with β-carotene assignment. According to the data availability of plasma antioxidants, the sample sizes for the interaction models were slightly reduced for individual antioxidants (511 cases and 423 controls for selenium; 541 cases and 642 controls for others) and the analysis for the antioxidant combination included 496 cases and 402 controls. Men with and without data on prediagnostic plasma antioxidant levels did not significantly differ in terms of baseline characteristics; we observed similar associations between MnSOD polymorphism and prostate cancer risk among men with and those without data on plasma antioxidant levels. All statistical analyses were conducted by using SAS (version 8.12, SAS Institute Inc., Cary, NC); all P values are two-sided.

Results

Table 1 presents the characteristics of prostate cancer cases and controls. Of men diagnosed with incident prostate cancer, 281 had nonaggressive disease and 275 had aggressive disease. The average interval from baseline in 1982 (when the blood was collected) to

Table 1. Baseline characteristics of prostate cancer case and control subjects enrolled in the Physicians' Health Study

Characteristic	Cases (n = 567)	Controls (n = 764)
Mean age at baseline ± SD (y)*	60.7 ± 7.6	60.8 ± 7.6
Mean age at diagnosis ± SD (y)	68.5 ± 6.7	
Smoking status (%)*		
Current	8.1	8.4
Former	46.0	46.6
Disease status (%)†		
Nonaggressive prostate cancer	49.6	
Aggressive prostate cancer	48.5	
Unknown	1.9	
MnSOD allele (%)		
Valine (V)	48.7	49.7
Alanine (A)	51.3	50.3
MnSOD polymorphism (%)		
MnSOD VV	132 (23.3)	190 (24.9)
MnSOD VA	288 (50.8)	379 (49.6)
MnSOD AA	147 (25.9)	195 (25.5)
Plasma antioxidant levels‡		
α-Carotene (ng/mL)	55.6 (26.4-132.0)	60.7 (24.9-148.5)
β-Carotene (ng/mL)	226.2 (96.1-488.1)	226.6 (98.6-520.9)
β-cryptoxanthin (ng/mL)	65.3 (26.4-148.2)	63.6 (28.8-146.1)
Lutein (ng/mL)	96.1 (51.3-224.8)	88.6 (50.1-193.7)
Lycopene (ng/mL)	385.9 (191.7-673.2)	392.5 (209.1-694.2)
α-Tocopherol (ng/mL)	11,174.8 (7,119.6-17,623.8)	10,878.9 (7,688.8-17,409.9)
γ-Tocopherol (ng/mL)	1,770.2 (978.8-2,949.9)	1,743.7 (1,025.0-2,922.3)
Retinol (ng/mL)	574.2 (408.4-797.4)	548.9 (394.9-751.5)
Selenium (ppm)	0.106 (0.084-0.130)	0.107 (0.084-0.131)
Receive β-carotene assignment (%)	48.3	52.5

*Matching variable.
†Aggressive prostate cancer, stage C or D, high-grade tumor (Gleason score 7-10), or prostate cancer deaths during the follow-up; nonaggressive prostate cancer, stage A or B and low grade (Gleason score 2-6) tumor.
‡Values are medians (10th and 90th percentile); antioxidant levels were available for 511 to 541 cases and 423 to 642 controls.

diagnosis was 8 years, and the median follow-up duration for these cases was 7 years. Plasma antioxidant levels of selenium, carotenoids, vitamin E, and retinol and the percentages of men who received β-carotene treatment were similar between cases and controls. The *MnSOD* allele frequencies among cases and controls were similar. Among control subjects, the genotype distributions were in accordance with Hardy-Weinberg equilibrium and the allele distribution (A allele, 50.3%) is similar to what was previously observed in Caucasians (5). Overall, MnSOD genotype was not associated with risk of total, nonaggressive, or aggressive prostate cancer (Table 2).

We found the MnSOD genotype significantly modified the association between prediagnostic plasma selenium status and prostate cancer risk ($P_{interaction}$ = 0.05 for total and 0.01 for aggressive prostate cancer; Table 3). In stratified analyses by the MnSOD genotype, plasma selenium levels were significantly and inversely associated with risks of total (4th versus 1st quartile RR, 0.33; 95% CI, 0.16-0.68, P_{trend} = 0.002) and aggressive (RR, 0.18; 95% CI, 0.07-0.48, P_{trend} < 0.001) prostate cancer among men homozygous for the A allele, whereas these inverse associations were weaker among VV/VA men. The results presented in Table 3 use a common reference group (i.e., men with the VV/VA genotype and antioxidant level in the lowest quartile). Compared with these men, we observed a significant increased risk of total and especially clinically aggressive (RR, 1.89; 95% CI, 1.01-3.56) prostate cancer for

Table 2. Association between MnSOD polymorphism and prostate cancer risk

Categories	MnSOD genotype		
	VV	VA	AA
Controls, *N*	190	379	195
All prostate cancer (567 cases)			
Cases, *N*	132	288	147
RR (95% CI)	1.00 (Reference)	1.09 (0.84-1.43)	1.09 (0.80-1.49)
By disease status*			
Nonaggressive prostate cancer (281 cases)			
Cases, *N*	67	143	71
RR (95% CI)	1.00 (Reference)	1.06 (0.75-1.49)	1.01 (0.68-1.50)
Aggressive prostate cancer (275 cases)			
Cases, *N*	64	139	72
RR (95% CI)	1.00 (Reference)	1.09 (0.77-1.54)	1.10 (0.74-1.62)

*Aggressive prostate cancer, stage C or D, high-grade tumor (Gleason score 7-10), or prostate cancer deaths during the follow-up; nonaggressive prostate cancer, stage A or B and low-grade (Gleason score 2-6) tumor; 11 cases with unknown disease status were excluded.

men with the AA genotype and selenium level in the lowest quartile; however, the RR was 0.35 (95% CI, 0.15-0.82; 5-fold difference) for those with the AA genotype and selenium level in the highest quartile. We found similar, although weaker, interactions between MnSOD polymorphism and plasma levels of lycopene or α-tocopherol. As expected, based on their lack of association with risk of prostate cancer, other carotenoids, γ-tocopherol, or retinol did not interact with the MnSOD polymorphism in modifying prostate cancer risk (data not shown).

Because plasma levels of selenium, lycopene, and α-tocopherol are each inversely associated with risk of prostate cancer (15–19), we further evaluated the associations of prostate cancer risk with the combined status of lycopene, α-tocopherol, and selenium and whether the MnSOD polymorphism modified this antioxidant combination in relation to prostate cancer risk. Based on the derived antioxidant score (Fig. 1), men with high (antioxidant score = 10-12) prediagnostic plasma antioxidant status had a RR of 0.60 (95% CI, 0.39-0.88, P_{trend} = 0.02) for total prostate cancer and 0.40 (95% CI, 0.25-0.69, P_{trend} = 0.002) for aggressive disease, compared with those with low antioxidant status (score = 3-5). The interaction between combined antioxidant score and MnSOD polymorphism ($P_{interaction} \leq 0.02$; Fig. 2) was stronger than those for antioxidants assessed individually. In stratified analyses by the genotype, among men homozygous for the A allele, high (versus low) antioxidant score was associated with significantly reduced risk of total (5-fold; RR, 0.20; 95% CI, 0.09-0.49) and aggressive prostate cancer (10-fold; RR, 0.10; 95% CI, 0.03-0.29); whereas the inverse association between the antioxidant score and prostate cancer risk was much weaker and not significant for those with the VV/VA genotype. Compared with the common reference group (AA men with low antioxidant status), men with the AA genotype and low antioxidant status had 2.5- and 3.1-fold increased risk for total (Fig. 2*A*) and aggressive (Fig. 2*C*) prostate cancer, respectively; however, the corresponding RR were 0.50 and 0.31 for the AA men who had high antioxidant status. The patterns for nonaggressive prostate cancer were similar but the interaction between MnSOD polymorphism and antioxidant status was not statistically significant. Because prostate cancers detected through prostate-specific antigen (PSA) screening may differ from clinically detected cases, we did all the above analyses for men diagnosed in the pre-PSA (1982-1990) and post-PSA (1991-1995) eras separately; results were similar (data not shown).

In the Physicians' Health Study trial, we previously reported no overall effect of β-carotene supplement on risk of cancer after 13 years (25, 26). We found no significant interaction between β-carotene assignment and the MnSOD polymorphism for risk of total or aggressive prostate cancer. However, β-carotene treatment (versus placebo) showed a significant protection against fatal prostate cancer (RR, 0.37; 95% CI, 0.15-0.94) among men with the AA (but not the VV/VA) genotype ($P_{interaction}$ = 0.03). Aspirin assignment was not associated with risk of prostate cancer, and we found no suggestion of an interaction between the MnSOD polymorphism and aspirin treatment in modifying prostate cancer risk.

Table 3. Relative risks and 95% CI for prostate cancer according to MnSOD genotype and quartiles of individual plasma antioxidant levels

	Quartile of prediagnostic plasma antioxidant level*				
	1	2	3	4	$P_{interaction}$
All prostate cancer					
Selenium (511 cases and 423 controls)					
MnSOD VV and VA	1.00 (Reference)	0.57	0.60	0.67	0.05
MnSOD AA	1.49	0.80	0.59	0.47 (0.26-0.85)†	
Lycopene (541 cases and 642 controls)					
MnSOD VV and VA	1.00 (Reference)	0.90	0.92	0.80	0.35
MnSOD AA	1.49	0.78	1.08	0.84 (0.51-1.38)†	
α-Tocopherol (541 cases and 642 controls)					
MnSOD VV and VA	1.00 (Reference)	1.16	1.17	1.14	0.13
MnSOD AA	1.78 (1.01-3.12)†	1.01	1.21	1.12 (0.67-1.88)†	
Aggressive prostate cancer‡					
Selenium (251 cases and 423 controls)					
MnSOD VV and VA	1.00 (Reference)	0.71	0.64	0.74	0.01
MnSOD AA	1.89 (1.01-3.56)†	1.17	0.59	0.35 (0.15-0.82)†	
Lycopene (261 cases and 642 controls)					
MnSOD VV and VA	1.00 (Reference)	0.78	0.94	0.70	0.23
MnSOD AA	1.73	0.69	0.72	0.79 (0.43-1.48)†	
α-Tocopherol (261 cases and 642 controls)					
MnSOD VV and VA	1.00 (Reference)	1.31	1.01	0.89	0.03
MnSOD AA	1.97 (1.02-3.79)†	1.20	1.01	0.72 (0.35-1.48)†	

NOTE: RR and 95% CI were calculated using unconditional logistic regression, adjusted for age at baseline, smoking status, and duration of follow-up.
*Based on each antioxidant, men were categorized into quartiles based on the quartile cutoff points among control subjects (batch-specific quartile cutoff points for all antioxidants except selenium).
†RR (95% CI).
‡Aggressive prostate cancer, stage C or D, high-grade tumor (Gleason score 7-10), or prostate cancer deaths during the follow-up.

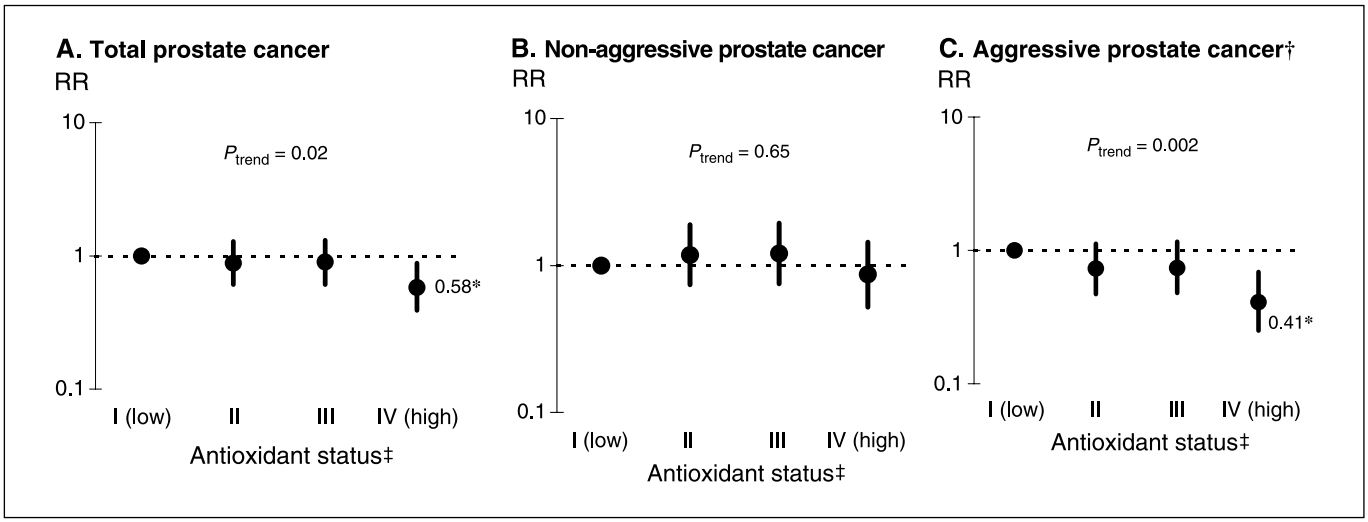

Figure 1. The association between prediagnostic plasma antioxidant combinations and risk of prostate cancer. †, Aggressive prostate cancer: stage C or D, high-grade tumor (Gleason score 7-10), or prostate cancer deaths during the follow-up; nonaggressive prostate cancer: stage A or B and low-grade (Gleason score 2-6) tumor; eight cases with unknown disease status were excluded. ‡, Antioxidant status: An antioxidant score (range, 3-12) was estimated based on quartile levels of plasma lycopene, α-tocopherol, and selenium among controls (batch-specific cutoff points for lycopene and α-tocopherol); four groups from low to high: *I,* antioxidant score = 3-5; *II,* antioxidant score = 6-7; *III,* antioxidant score = 8-9; and *IV,* antioxidant score = 10-12. Median antioxidant levels for groups I to IV: lycopene (ng/mL): 271.7, 355.4, 4,42.8, 581.5; α-tocopherol (ng/mL): 8,559.9, 9,893.3, 12,714.5, 14,428.8; selenium (ppm): 0.092, 0.104, 0.107, 0.120. *Dots,* RR; *bars*, CI. *A,* total prostate cancer: 496 cases and 402 controls; *95% CI, 0.39-0.88. *B,* nonaggressive prostate cancer: 264 cases and 402 controls. *C,* aggressive prostate cancer: 242 cases and 402 controls; *95% CI, 0.25-0.69.

Discussion

In this study, we found that men with the AA genotype for MnSOD were particularly sensitive to antioxidant status, with a 5-fold gradient in risk for aggressive prostate cancer across the four quartiles of plasma selenium levels (Table 3). In contrast, those with the *V* allele were much less sensitive to differences in selenium. These patterns were similar for prediagnostic plasma levels of lycopene and α-tocopherol and were particularly strong for these antioxidants combined (a 10-fold difference in risk between high versus low antioxidant group; Fig. 2*C*). Although intervention studies including the Physicians' Health Study indicate that β-carotene supplementation does not lower prostate cancer risk (26–28), except possibly in men with low β-carotene status at baseline (25), we found that β-carotene treatment was significantly protective for fatal prostate cancer for men with the AA genotype.

MnSOD as an endogenous antioxidant in mitochondria may play a role in preventing prostate cancer. Malignant prostate epithelium has lower MnSOD expression than benign prostatic epithelium (29) and overexpression of MnSOD in the prostate inhibits cancer cell growth *in vivo* (3). However, several recent papers reported an increased expression of MnSOD in advanced cancer tissue, particularly in relation to poor prognosis or metastasis (30–32). Although the function of the MnSOD polymorphism is not fully

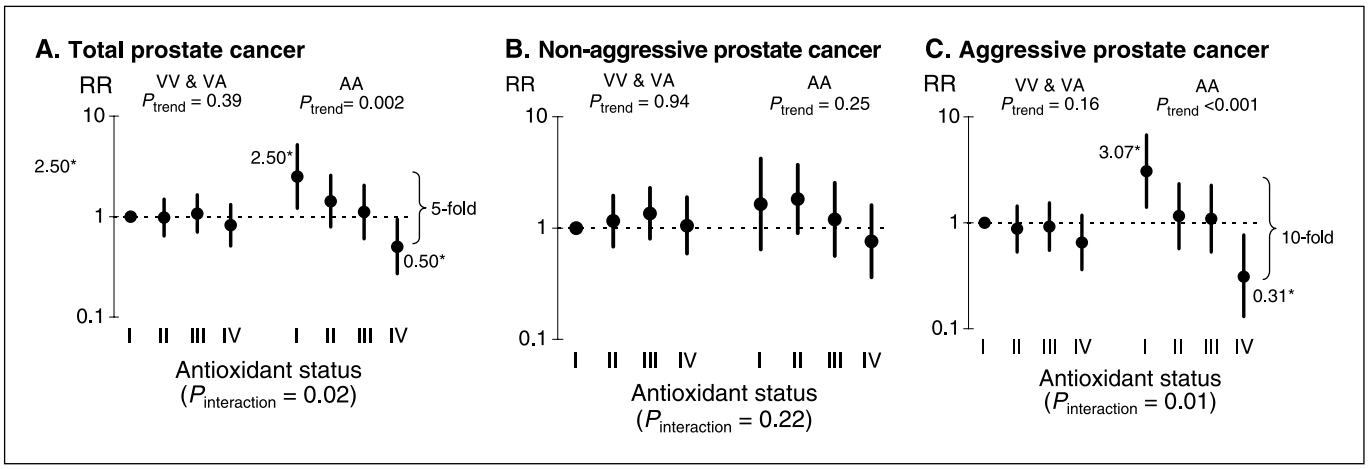

Figure 2. Risk of prostate cancer associated with MnSOD genotype modified by prediagnostic levels of plasma antioxidant combinations. Definitions for aggressive and nonaggressive prostate cancer and methods for categorizing men into four antioxidant groups were the same as in Fig. 1. *Dots,* RR; *bars,* 95% CI. *A,* total prostate cancer: 496 cases and 402 controls; *95% CI (for group I) = 1.21-5.20; 95% CI (for group IV) = 0.27-0.94; among AA men, group IV versus I RR, 0.20, 95% CI, 0.09-0.49. *B,* nonaggressive prostate cancer: 246 cases and 402 controls. *C,* aggressive prostate cancer: 242 cases and 402 controls; * 95% CI (for group I) = 1.40 to 6.76; 95% CI (for group IV) = 0.13-0.77; among AA men, group IV versus I RR, 0.10, 95% CI, 0.03-0.29.

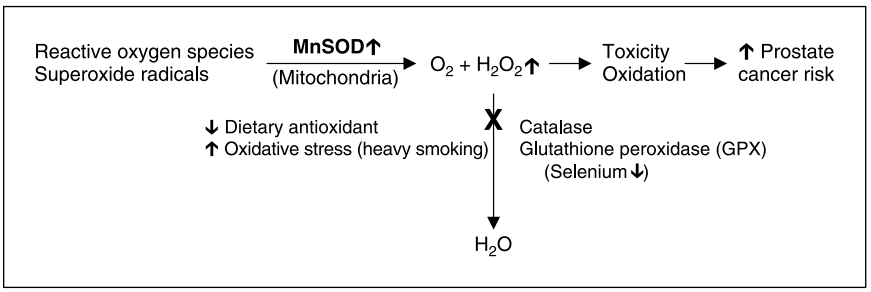

Figure 3. Potential mechanism for the interaction between MnSOD and antioxidant status.

understood, a recent study showed that the A-containing MnSOD is transported more efficiently through the mitochondrial membrane (6), suggesting that, compared with those with the VV/VA genotype, the individuals with the homozygous AA genotype may have higher MnSOD activity. In mitochondria, the superoxide anion is dismutated by MnSOD into oxygen and hydrogen peroxide (H_2O_2), which is further detoxified by mitochondrial glutathione peroxidase, an enzyme requiring selenium, or catalase into water (Fig. 3). Thus, high levels of MnSOD expression may lead to enzyme imbalance and induce toxicity if glutathione peroxidase activity is low due to inadequate selenium intake or if antioxidants are in high demand due to physiologic conditions or lifestyle factors, such as smoking (33). Hong et al. (34) reported that, among pregnant women in Korea, MnSOD variant AA was significantly related to increased formation of urinary 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative DNA damage.

The sensitivity of those with the AA genotype to antioxidants in our study is consistent with the observations from the Finnish prostate cancer study and studies of breast cancer and the MnSOD polymorphism. The Finnish study of male heavy smokers (7) found that the MnSOD AA polymorphism was significantly associated with increased risk of prostate cancer, especially high-grade tumors. Because of the low soil selenium content and probably low tomato (lycopene) consumption in Finland, the Finnish participants were likely comparable with the low antioxidant group in our study. Increased risk of breast cancer associated with the AA genotype in several studies among women with low intakes of antioxidants (5, 11) also agree with our findings. Because few participants were current smokers in our study, we were unable to assess the interaction of MnSOD polymorphism with smoking status.

Strengths of the current study included its prospective design, large sample size, and complete long-term follow-up. Prospectively collected plasma enabled us to assess the interaction of the MnSOD polymorphism with a variety of antioxidants, the combined antioxidant status, and β-carotene supplementation. Although a single assessment of antioxidants from the baseline blood is an imperfect indicator of long-term dietary exposures, significant findings on the associations of prostate cancer risk with plasma levels of selenium (15, 16), lycopene (17, 18), and α-tocopherol (17, 19), as well as the combined plasma antioxidant score in the current study, provide some assurance that these plasma antioxidant data are valid in reflecting long-term antioxidant status in our study population. Also, our simple antioxidant score may not be ideal to summarize overall antioxidant status. Our observational findings should be tested in randomized trials, such as the ongoing Selenium and Vitamin E Cancer Prevention Trial (35), and our findings for β-carotene supplementation could be further evaluated by other existing β-carotene intervention trials.

In this large prospective study of prostate cancer, our data provide strong and biologically plausible evidence that the association between antioxidant status and risk of prostate cancer is at least partially dependent on MnSOD genotype and that endogenous and exogenous antioxidants are both important for clinically significant prostate cancer. This interaction may well extend to other conditions where oxidative status is important.

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