Polymorphisms in Oxidative Stress–Related Genes Are Not Associated with Prostate Cancer Risk in Heavy Smokers

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Abstract

Oxidative stress, associated with aging and inflammation, is likely to play a role in the etiology of prostate cancer. We evaluated potential associations between gene variants that result in reduced neutralization of reactive oxygen species (ROS; *MnSOD* Ala-*16*Val, *CAT* −262 C>T, and *GPX1* Pro*200*Leu) and prostate cancer risk among 724 men with incident prostate cancer who participated in the Carotene and Retinol Efficacy Trial (CARET) cohort, a randomized trial for the prevention of lung cancer among men with a history of smoking and/or asbestos exposure. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by logistic regression. Nested case-control analyses included study participants with available DNA (n = 533 cases and 1,470 controls), matched for race, age, and length of follow-time. Overall, there were no associations between genotypes of *MnSOD*, *CAT*, and *GPX1* and prostate cancer risk, although among men diagnosed before age 65, *CAT* TT genotype was associated with increased risk (OR, 2.0; 95% CI, *0.97*-3.95). Further analyses stratified by factors related to environmental oxidative stress exposures did not modify associations. When calculating the number of risk alleles of *MnSOD, CAT*, and *GPX1* hypothetically related to reduced protection against ROS, there was a nonsignificant relationship between prostate cancer and carriage of five or more risk alleles, in comparison to men with less than five risk alleles (OR, 2.0; 95% CI, 0.90-*4.42*). In conclusion, it does not seem that variants in *MnSOD, CAT*, or *GPX1* have an influence on prostate cancer risk in this cohort of men who were smokers or exposed to asbestos, although it is possible that cumulative defects in protection from oxidative stress may result in increased risk of the disease. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1115–20)

Introduction

Although the absolute number of deaths from prostate cancer has been decreasing substantially over the past decade, it remains one of the most common cancers in the United States, representing approximately one-third of all cancers diagnosed among American men in 2006 (1). Understanding factors contributing to carcinogenesis in the prostate has been, and continues to be, a crucial element in improving methods of cancer prevention as a whole. Given that prostate cancer incidence is highly dependent upon age, correlations between aging and tumorigenesis demand greater attention, particularly the role played by oxidative damage in both processes (2-5).

The enzymes that are generally considered to be the front-line defense against reactive oxygen species (ROS) are the mitochondrial manganese superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPX1). MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, whereas CAT and GPX1 facilitate the further reduction of hydrogen peroxide to water and oxygen. By this chain of enzymatic events, most of the ROS in the cell are eliminated, and potential damage is limited.

A valine (Val)-to-alanine (Ala) substitution at amino acid 16 (T to C) occurs in the mitochondrial targeting sequence of the *MnSOD* gene (rs4880), which may affect the localization and transport of the enzyme into the mitochondria by altering the secondary structure of the protein (6). Sutton et al. (7) reported that *MnSOD* C alleles resulted in 30% to 40% greater efficiency in localizing the enzyme to the mitochondrial matrix, compared with *MnSOD* T alleles. Two studies, to date, have examined the influence of this polymorphism on the risk of prostate cancer (8, 9); one study observed a moderate elevation in risk, particularly among men with high-grade tumors (8). The other study found no overall association with risk, but the polymorphism significantly increased the risk of prostate cancer among men with lower prediagnostic levels of plasma antioxidants (9).

To our knowledge, there have been no investigations of associations between prostate cancer risk and *CAT* and *GPX1* polymorphisms. These variants have been linked to breast cancer and other disease conditions related to oxidative stress. A common −262 C to T (rs1001179) polymorphism has been identified in the promoter region of the human catalase gene (*CAT*), and individuals with variant CT or TT genotypes have significantly lower activity than those with CC genotypes in Caucasians (10). A Pro-to-Leu allele polymorphism (rs1050450) of selenium-dependent *GPX1* exists at codon 200 (C>T), with the variant Leu allele being less responsive than the common Pro allele to the stimulation of enzyme activity during selenium supplementation.

It is likely that the balance of oxidants and antioxidants is affected by numerous genetic variants, as well as endogenous and exogenous exposures. Because ultimate levels of ROS are likely dependent not only on the generation of hydrogen peroxide by MnSOD, but also on the neutralization of H_2O_2 by catalase and glutathione peroxidase, we evaluated potential associations between risk and variants in these enzymes, as well as potential interactions with ROS-related exposures, in a nested case-control study conducted in the β-Carotene and Retinol Efficacy Trial (CARET) study.

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Materials and Methods

Study Population. The CARET study was a multicenter randomized, double-blind, placebo-controlled chemoprevention trial to test β-carotene with or without vitamin A (retinol) against a placebo for the prevention of lung cancer among 18,314 heavy smokers and asbestos-exposed workers. Participant accrual for the intervention began in 1985, and the study ended in 1996, when interim analysis found evidence that the supplements increased the risk of lung cancer and total mortality (11). Active follow-up of all participants continued until 2005 and included the collection of end point data. Age, sex, race/ethnicity, education, smoking history, alcohol use, general health history, and body mass index (BMI) were collected at each participant's first CARET clinical visit. Blood was collected in foil-covered Vacutainers at the time of the study center visits and was aliquotted and frozen for later analysis. Other details about the design and primary results of CARET have been previously published (11-13). The Institutional Review Board of the Fred Hutchinson Cancer Research Center and each of the five other participating institutions approved all procedures for the study, and participants provided written informed consent at recruitment and throughout the study. For this report, additional Institutional Review Board approval was obtained from Roswell Park Cancer Institute.

Prostate Cancer Cases—End Points Collection. When a cancer end point was reported, the medical records and pathology reports were obtained from the diagnosing physician/hospital and abstracted for stage, histology, and Gleason score by one of the co-authors (G.G.). A total of 778 cases were confirmed through 2003 and considered for a series of nested case-control studies, including the current study. Excluded were those with a diagnosis of prostate cancer before their baseline visit ($n = 9$), a diagnosis of any other primary cancer (except nonmelanoma skin cancer) on or before the date of prostate cancer diagnosis ($n = 41$), or insufficient serum and DNA for laboratory analyses ($n = 19$). After exclusions, 709 cases were eligible for the study. Fifteen participants initially selected as controls were later diagnosed with prostate cancer and are treated as cases in statistical analyses, bringing the total number of cases to 724. The Cancer Surveillance System of Western Washington, which is part of the National Cancer Institute's SEER registry, was used to obtain stage and Gleason scores for 46 of 143 and 34 of 84 participants, respectively, for whom these data were not available from the participant's medical records. Thus, staging information and Gleason scores were available for 627 (87%) and 674 (93%) of the study cases. A man was considered as having aggressive prostate cancer if he was diagnosed with extraprostatic extension or metastasis (stage III or IV) or with Gleason sum of ≥7.

The current study was restricted to 533 cases with genotyping data available. The reduced sample size for this analysis is due to the one-time collection of whole blood (from which DNA was extracted for genotyping) at 4 years post-baseline on average. Overall, whole blood was collected on 68% of CARET participants.

Control Section for Nested Case-Control Studies. For the nested case-control study, men were eligible for the control group if they were free of both prostate cancer and lung cancer (the primary end point in CARET) and had available baseline serum and whole blood, or extracted DNA, in the CARET repository. Cases and controls were matched on age (5-year increments), race/ethnicity, and follow-up time in CARET. It was required that the minimum follow-up time for the controls was equivalent to the time at diagnosis for the matched cases. We targeted a case-control ratio of 1:4 for all African Americans and 1:2 for other races, resulting in a total of 724 cases and 1,474 controls (after reassigning the 15 participants who were originally selected as controls and diagnosed subsequently with prostate cancer). Genotyping results were available for all but four controls (for whom genotyping failed). Thus, for the present study, a total of 1,470 controls and 533 cases, as described in the previous section, were available for statistical analysis.

DNA Extraction and Genotyping. Genomic DNA was extracted from whole blood samples with the use of QIAamp DNA blood Midi kits (Qiagen), and genotyping was done by BioServe Biotechnologies using Sequenom's high-throughput matrix-assisted laser desorption/ionizing time-of-flight mass spectrometry as previously described (14, 15). There was excellent observer agreement in the 8% of randomly selected duplicates of genotyping results that were included for quality control purposes (κ statistic, 0.95), with <1% assay failure rate.

Statistical Analysis. Genotype distributions in the controls were tested for Hardy-Weinberg equilibrium to evaluate possible selection bias and genotyping errors. The risk of prostate cancer was estimated with odds ratios (OR) and 95% confidence intervals (95% CI), using unconditional logistic regression models. In multivariate analyses, the ORs for genotypes or risk factors of interest were adjusted for age at enrollment (continuous), family history of prostate cancer in first-degree relatives (yes or no), CARET randomization assignment (intervention or placebo), cigarette smoking status (never or former and current at baseline), pack-years smoked (<40, 40–60, and >60), alcohol consumption (nondrinker, below median, and at or above median based on median of total alcohol amount in controls), and vitamin supplement use (yes or no). Variables included for adjustment were those that were significant covariates in the initial logistic regression model ($P < 0.2$) and were covariates in the previous CARET studies. Additional variables that were initially tested but not included in the final models were education (<12 years, high school graduate, some college, and 4-year college degree or higher) and BMI (normal, <25.0; overweight, 25.0–29.9; and obese, ≥30.0). Tests for linear trend in risk were conducted by treating categorical values as a continuous variable.

Associations between genotype and risk were examined among subgroups of cases according to age at diagnosis (<65 years versus ≥65 years) and disease status (nonaggressive prostate cancer versus aggressive prostate cancer) using polychotomous regression models, adjusting for the covariates listed above. To explore whether the main effects of genotypes were modified by oxidative stress–related exposures, associations between prostate cancer risk and variant alleles of *MnSOD*, *CAT*, and *GPX1* were examined among categories of vitamin supplement use (yes versus no), alcohol consumption (never and below median versus above median), BMI (<30.0 versus ≥30), cigarette smoking status (never or former versus current at baseline) and pack-years smoked (≤60 versus >60).

Potential interactions between genotypes and risk factors or prognostic factors were evaluated by the likelihood ratio test. The difference of two $-2\text{Log}L$ values of logistic models with and without cross-product terms of the ordinal score of each genotype and the risk factor variables stratified (e.g., genotype × vitamin supplement) were tested by χ^2, with one degree of freedom.

To evaluate whether multiple single nucleotide polymorphisms (SNP) in the same pathway have synergistic or additive effects on prostate cancer risk, we conducted additional exploratory analyses. ''High-risk'' alleles of each gene were categorized based on prior associations in the literature, and the total numbers of risk alleles for each individual were tallied. The number of risk alleles for each individual ranged from 0 to 6; associations with prostate cancer risk were evaluated for each group based on the number of risk alleles. In a post hoc analysis, we further dichotomized categories based on distributions in cases and controls into those with fewer than five risk alleles set as

reference group. All statistical analyses were done using STATA version 9.0 (Stata Corporation). Power calculations were carried out using Power (16, 17).

Results

Prostate cancer cases and controls were similar in age, race, and random assignment to the CARET intervention. Approximately 60% of the subjects were age 60 years or older, 90% were Caucasian, half were assigned to the intervention trial, half were current smokers at randomization, and almost 30% were obese. Of men diagnosed with prostate cancer, 269 had nonaggressive disease, 249 had aggressive disease, and 15 had no information. History of prostate cancer in a first-degree relative (OR, 2.0; 95% CI, 1.25-3.30) and alcohol consumption (OR, 1.5; 95% CI, 1.13-1.93 for at or above

Table 1. Association between *MnSOD*, *CAT*, and *GPX1* genetic polymorphisms and risk of prostate cancer

	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)*
Overall						
MnSOD						
TT	128 (25.5)	356 (25.7)	1.0 (ref)			
TC	258 (51.4)	683 (49.2)	1.1 (0.83-1.39)			
CC	116 (23.1)	349 (25.1)	0.9 (0.68-1.27)			
CAT						
CC	317 (62.4)	885 (63.1)	1.0 (ref)			
CT	165 (32.5)	461 (32.9)	1.0 (0.77-1.22)			
TT	26 (5.1)	57 (4.1)	1.2 (0.75-2.06)			
GPX1						
CC	249 (49.8)	704 (50.6)	1.0 (ref)			
CT	213 (42.6)	578 (41.6)	1.0 (0.80-1.25)			
TT	38 (7.6)	109 (7.8)	1.0 (0.64-1.47)			
Race †	Caucasian			African American		
MnSOD						
TT	112 (24.6)	293 (24.1)	1.0 (ref)	7 (25.0)	39 (32.0)	1.0 (ref)
TC	239 (52.5)	610 (50.3)	1.0 (0.79-1.37)	15 (53.6)	52 (42.6)	2.1 (0.67-6.31)
CC	104 (22.9)	311 (25.6)	0.9 (0.63-1.21)	6 (21.4)	31 (25.4)	1.3 (0.34-5.08)
CAT						
CC	281 (60.7)	732 (59.4)	1.0 (ref)	24 (88.9)	109 (90.8)	1.0 (ref)
CT	157 (33.9)	445 (36.1)	0.9 (0.71-1.15)	3 (11.1)	11 (9.2)	1.4 (0.32-6.42)
TT	25 (5.4)	56 (4.5)	1.1 (0.68-1.93)	—	—	—
GPX1						
CC	227 (50.2)	616 (50.5)	1.0 (ref)	12 (41.4)	51 (42.9)	1.0 (ref)
CT	190 (40.0)	515 (42.2)	1.0 (0.77-1.23)	15 (51.7)	53 (44.5)	1.1 (0.42-2.73)
TT	35 (7.7)	90 (7.4)	1.1 (0.68-1.64)	2 (6.9)	15 (12.6)	0.4 (0.06-1.96)
Randomization assignment ‡	Placebo			Intervention		
MnSOD						
TT	52 (24.2)	148 (25.0)	1.0 (ref)	60 (25.0)	145 (23.3)	1.0 (ref)
TC	114 (53.0)	297 (50.2)	1.0 (0.66-1.43)	125 (52.1)	313 (50.3)	1.1 (0.75-1.66)
CC	49 (22.8)	147 (24.8)	0.9 (0.55-1.38)	55 (22.9)	164 (26.4)	0.9 (0.56-1.43)
CAT						
CC	136 (62.7)	351 (58.6)	1.0 (ref)	145 (60.1)	381 (60.1)	1.0 (ref)
CT	70 (32.3)	220 (36.7)	1.0 (0.73-1.42)	87 (35.4)	225 (35.5)	0.8 (0.57-1.14)
TT	11 (5.1)	28 (4.7)	1.2 (0.56-2.42)	14 (5.7)	28 (4.4)	1.1 (0.53-2.36)
GPX1						
CC	114 (52.8)	308 (51.9)	1.0 (ref)	113 (47.9)	308 (49.1)	1.0 (ref)
CT	81 (37.5)	237 (39.9)	1.0 (0.74-1.42)	109 (46.2)	278 (44.3)	0.9 (0.64-1.28)
TT	21 (9.7)	49 (8.3)	0.9 (0.47-1.87)	14 (5.9)	41 (6.5)	1.1 (0.61-1.98)
	Controls	Cases	OR (95% CI)§	Cases	OR (95% CI)§	
Age at diagnosis (y) ‡	<65			≥65		
MnSOD						
TT	293 (24.1)	45 (27.0)	1.0 (ref)	67 (23.3)	1.0 (ref)	
TC	610 (50.3)	88 (52.7)	1.0 (0.64-1.53)	151 (52.4)	1.1 (0.78-1.54)	
CC	311 (25.6)	34 (20.3)	0.8 (0.48-1.37)	70 (24.3)	0.9 (0.63-1.41)	
CAT						
CC	732 (59.4)	95 (54.6)	1.0 (ref)	186 (64.4)	1.0 (ref)	
CT	445 (36.1)	66 (37.9)	1.1 (0.74-1.57)	91 (31.5)	0.8 (0.59-1.07)	
TT	56 (4.5)	13 (7.5)	2.0 (0.97-3.95)	12 (4.2)	0.7 (0.35-1.50)	
GPX1						
CC	616 (50.5)	90 (52.6)	1.0 (ref)	137 (48.8)	1.0 (ref)	
CT	515 (42.2)	69 (40.4)	1.0 (0.68-1.44)	121 (43.1)	0.9 (0.71-1.27)	
TT	90 (7.4)	12 (7.0)	1.0 (0.49-1.93)	23 (8.2)	1.1 (0.63-1.87)	

*ORs adjusting for age at enrollment, history of prostate cancer in first-degree relative, random assignment, smoking status (current versus former or nonsmoker), pack-years smoked (<40, 40-60, ≥60), alcohol consumption (nondrinker, below median, at or above median) and vitamin supplement use in logistic regression model.
†P = 0.133 for *MnSOD*, <0.001 for *CAT*, and 0.075 for *GPX1* between Caucasian and African American tested by χ^2 test in controls.
‡Restricted to Caucasians only.
§ORs adjusting for age at enrollment, history of prostate cancer in first-degree relative, random assignment, smoking status (current versus former or nonsmoker), pack-years smoked (<40, 40-60, ≥60), alcohol consumption (nondrinker, below median, at or above median) and vitamin supplement use in polychotomous logistic regression model.

median alcohol amount compared with nondrinker; P_{trend} = 0.002) were associated with increased prostate cancer risk, and the use of vitamin supplements at baseline was associated with decreased risk (OR, 0.8; 95% CI, 0.63-0.98). There were no differences in other participant characteristics between cases and controls. There were no significant differences between individuals with genotyping data and those included in the larger nested case-control study in any of the variables assessed except for alcohol consumption ($P < 0.001$ by χ^2 test).

The frequencies of variant alleles in *MnSOD* (C), *CAT* (T), and *GPX1* (T) in controls were 0.50, 0.23, and 0.28 in Caucasians (n = 1,293), and 0.46, 0.05, and 0.34 in African Americans (n = 125), respectively (Table 1). All genotypes were in Hardy-Weinberg equilibrium in both Caucasians and in African Americans. The distributions of genotypes between control Caucasians and African Americans were significantly different in *CAT* ($P < 0.001$) but not *MnSOD* (P = 0.131) and *GPX1* (P = 0.131), evaluated by χ^2 test. Because the numbers of African American men were too low to perform analyses stratified by race and because of the differential distribution of alleles by race, additional analyses were limited to Caucasians only.

Associations between genotypes and risk of prostate cancer, overall and stratified by potential modifying variables, are shown in Table 1. There were no overall associations between risk and polymorphisms in *MnSOD*, *CAT*, and *GPX1*. However, among men diagnosed before age 65, there was more than a 2-fold increase in risk for those with *CAT* TT genotypes (OR, 2.0; 95% CI, 0.97-3.95; P_{trend} = 0.079). There were no significant associations between genotypes and risk among any other strata, including race, randomization assignment, and age at diagnosis (Table 1). We also tested the associations stratified by disease status, but the genotypes were not associated with nonaggressive or aggressive prostate cancer (data not shown).

Biological effects of genotypes may only be apparent among those with high levels of ROS either due to exogenous exposures, endogenous factors, or from fat-mass–induced generation. Therefore, we examined potential modification by stratifying data by vitamin supplement use, alcohol consumption, BMI, smoking status, and pack-years smoked. There was a significant increase in risk with *MnSOD* TT genotypes (OR, 2.1; 95% CI, 1.23-3.47) among those who never used supplements compared with supplement users with *MnSOD* TT genotypes (Table 2). There were no significant interactions between supplement use and other genotypes. Smoking status and BMI did not seem to modify any genotype-prostate cancer risk associations. There were also no significant differences between strata of alcohol consumption or pack-years smoked (data not shown).

Table 2. Association between *MnSOD*, *CAT*, and *GPX1* genetic polymorphisms and risk of prostate cancer stratified by ROS exposures in Caucasians

	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)*
Vitamin supplement		Yes			No	
MnSOD						
TT	29 (18.0)	114 (24.4)	1.0 (ref)	83 (28.2)	179 (24.0)	2.1 (1.23-3.47)
TC	93 (57.8)	228 (48.8)	1.7 (1.05-2.88)	146 (49.7)	381 (51.1)	1.7 (1.03-2.73)
CC	39 (24.2)	125 (26.8)	1.3 (0.72-2.30)	65 (21.1)	186 (24.9)	1.5 (0.89-2.60)
CAT						
CC	93 (57.1)	293 (61.4)	1.0 (ref)	188 (62.7)	439 (58.2)	1.4 (1.03-1.88)
CT	59 (36.2)	158 (33.1)	1.1 (0.75-1.66)	98 (32.7)	286 (37.9)	1.1 (0.79-1.57)
TT	11 (6.8)	26 (5.5)	1.2 (0.56-2.71)	14 (4.7)	30 (4.0)	1.5 (0.76-3.14)
GPX1						
CC	83 (51.2)	238 (50.6)	1.0 (ref)	144 (49.7)	377 (50.3)	1.1 (0.80-1.55)
CT	73 (45.1)	198 (42.1)	1.0 (0.69-1.48)	117 (40.3)	317 (42.3)	1.1 (0.75-1.49)
TT	6 (3.7)	34 (7.2)	0.4 (0.16-1.13)	29 (10.0)	56 (7.5)	1.6 (0.95-2.82)
Smoking status		Never or former			Current	
MnSOD						
TT	52 (23.2)	145 (25.5)	1.0 (ref)	60 (26.0)	148 (23.0)	1.0 (0.65-1.62)
TC	119 (53.1)	291 (51.1)	1.1 (0.77-1.70)	120 (52.0)	319 (49.5)	1.0 (0.65-1.44)
CC	53 (23.7)	133 (23.4)	1.0 (0.62-1.62)	51 (22.0)	178 (27.5)	0.8 (0.49-1.24)
CAT						
CC	138 (60.0)	348 (60.3)	1.0 (ref)	143 (61.4)	384 (58.5)	0.9 (0.66-1.18)
CT	77 (33.5)	200 (34.7)	1.0 (0.68-1.35)	80 (34.3)	245 (37.4)	0.8 (0.53-1.06)
TT	15 (6.5)	29 (5.0)	1.2 (0.61-2.49)	10 (4.3)	27 (4.1)	0.9 (0.44-2.04)
GPX1						
CC	116 (51.8)	278 (48.6)	1.0 (ref)	111 (48.7)	338 (52.1)	0.7 (0.54-1.03)
CT	92 (41.1)	249 (43.5)	0.9 (0.63-1.23)	98 (43.0)	266 (41.0)	0.8 (0.57-1.12)
TT	16 (7.1)	45 (7.9)	0.8 (0.40-1.47)	19 (8.3)	45 (6.9)	1.0 (0.58-1.92)
BMI		<30.0			≥30.0	
MnSOD						
TT	82 (24.7)	205 (23.4)	1.0 (ref)	30 (25.0)	87 (26.3)	0.9 (0.52-1.47)
TC	176 (53.0)	449 (51.3)	1.0 (0.72-1.38)	62 (51.7)	158 (47.7)	1.0 (0.67-1.51)
CC	74 (22.3)	221 (25.3)	0.8 (0.57-1.24)	28 (23.3)	86 (26.0)	0.8 (0.47-1.36)
CAT						
CC	205 (61.2)	526 (59.6)	1.0 (ref)	75 (60.0)	201 (58.8)	1.0 (0.70-1.34)
CT	112 (33.4)	316 (35.8)	0.9 (0.68-1.20)	43 (34.4)	126 (36.8)	0.8 (0.56-1.25)
TT	18 (5.4)	41 (4.6)	1.1 (0.57-1.96)	7 (5.6)	15 (4.4)	1.4 (0.53-3.51)
GPX1						
CC	166 (51.2)	445 (50.8)	1.0 (ref)	60 (48.0)	168 (49.8)	1.0 (0.70-1.34)
CT	136 (42.0)	366 (41.8)	1.0 (0.75-1.31)	52 (41.6)	146 (43.3)	0.9 (0.56-1.25)
TT	22 (6.8)	65 (7.4)	0.8 (0.48-1.46)	13 (10.4)	23 (6.8)	1.7 (0.53-3.51)

*ORs adjusting for age at enrollment, history of prostate cancer in first-degree relative, random assignment, smoking status (current versus former or nonsmoker), pack-years smoked (<40, 40-60, ≥60), alcohol consumption (nondrinker, below median, at or above median) and vitamin supplement use in logistic regression model.

Table 3. Combination of *MnSOD, CAT*, and *GPX1* genetic polymorphisms and risk of prostate cancer in Caucasian

Number of risk alleles*	Cases	Controls	Crude OR (95% CI)	OR (95% CI) †
0	31 (7.4)	85 (7.6)	1.0 (ref)	1.0 (ref)
1	117 (28.1)	287 (25.7)	1.1 (0.70-1.78)	1.1 (0.69-1.85)
2	140 (33.6)	368 (33.0)	1.0 (0.66-1.64)	1.0 (0.59-1.56)
3	87 (20.9)	274 (24.5)	0.9 (0.54-1.40)	0.8 (0.51-1.41)
4	30 (7.2)	86 (7.7)	1.0 (0.53-1.72)	1.0 (0.52-1.81)
≥5	12 (2.8)	17 (1.5)	1.9 (0.83-4.51)	1.9 (0.79-4.80)
0–4	405 (97.2)	1,100 (98.5)	1.0 (ref)	1.0 (ref)
≥5	12 (2.8)	17 (1.5)	1.9 (0.91-4.05)	2.0 (0.90-4.42) ‡

NOTE: The subjects with six risk alleles were only two.
*Risk alleles were defined as *MnSOD* C allele, *CAT* T allele, and *GPX1* T allele reducing protection against ROS.
†ORs adjusting for age at enrollment, history of prostate cancer in first-degree relative, random assignment, smoking status (current versus former or nonsmoker), pack-years smoked (<40, 40-60, ≥60), alcohol consumption (nondrinker, below median, at or above median) and vitamin supplement use in logistic regression model.
‡$P = 0.091$.

When calculating the number of risk alleles of *MnSOD, CAT*, and *GPX1* (reducing protection against ROS, *MnSOD* C allele, *CAT* T allele, and *GPX1* T), men with five or six risk alleles had marginally significantly increased risk of prostate cancer compared with men with less than five risk alleles (OR, 2.0; 95% CI, 0.90-4.42; Table 3). Although risk with five or more risk alleles seemed to be greatest among those who were higher consumers of alcohol (OR, 3.1; 95% CI, 0.97-9.73) and who were obese (BMI ≥ 30.0; OR, 5.2; 95% CI, 0.94-29.00), relationships were not statistically significant.

Discussion

Risk of prostate cancer increases with age, indicating that inflammatory processes and cumulative exposure to ROS over the life course could be related to carcinogenesis in the prostate. Previous studies found that patients with prostate cancer had lower expression of antioxidant enzymes (i.e., MnSOD, CAT, and GPX1) than men with benign disease or healthy controls (2-5) and noted that DNA damage in prostate cancer tissue from aging males seems to be the result of oxidative damage induced by hydroxyl radicals (18, 19). Recently, Lockett et al. (20) found that hydrogen peroxide–induced DNA damage in lymphocytes was associated with a 1.6-fold increase in prostate cancer risk, and relationships seemed to be modified by exogenous sources of ROS, such as smoking history and BMI.

We evaluated relationships between risk of prostate cancer and polymorphisms in genes relevant to oxidative stress (*MnSOD* Ala16Val, *CAT* −262 C>T, and *GPX1* Pro200Leu), as well as potential modification of associations by exposure to various sources of ROS, among men who were participants in the CARET study. Although some associations were noted in stratified analysis, we did not observe significant relationships between any genotypes and prostate cancer risk, nor any consistent interactions with potential modifiers of relationships. Our study had >90% power to detect an OR of 1.5 for SNPs of *MnSOD, CAT*, and *GPX1* with the allele frequency in this study. The allele frequencies between our study and previous studies (9, 14, 21) were similar for all three SNPs among the healthy populations.

The C allele–containing MnSOD is transported more efficiently through the mitochondrial membrane (7), suggesting that individuals with homozygous CC genotypes may have higher MnSOD activity compared with those with TT/TC genotypes. Because MnSOD removes the superoxide anion, a potential source of DNA damage, one would predict that the *MnSOD* C allele would lead to a decreased risk of cancer. On the other hand, MnSOD also generates hydrogen peroxide that can be toxic if not removed; thus, the C allele could be associated with increased risk of cancer. Indeed, studies of cancer in hormone-dependent organs (i.e., breast, ovary, and prostate) have observed increased risk with the C allele (8, 9, 22-24); however, studies of other cancer sites (i.e., bladder, lung, and colorectal) have indicated increased risk with T alleles (25-27). Two studies, to date, have examined the influence of this polymorphism on the risk of prostate cancer (8, 9). Woodson et al. (8) reported that C alleles were associated with increased risk of prostate cancer in a cohort of Finnish male smokers, with risk elevated among men with high-grade tumors, although sample size was limited. Finnish participants live in an area with low soil selenium; thus, antioxidant levels from diet could be quite low, and endogenous oxidants and antioxidants are more important. In the Physicians' Health Study, Li et al. (9) observed little overall association between *MnSOD* variants and prostate cancer risk, consistent with our findings. However, higher levels of plasma antioxidants (selenium, lycopene, and α-tocopherol) were associated with decreased risk only among men with CC genotypes (versus TT/TC genotype).

This study is the first to evaluate the *CAT* polymorphism in relation to prostate cancer risk. In a case-control study of breast cancer, we (CA) previously found that *CAT* CC genotypes encoding higher activity levels were associated with a 17% reduction in risk, which was most notable among higher consumers of fruits and vegetables (10, 14). Although no main effect of *CAT* genotypes was observed overall in relation to prostate cancer risk, we found that *CAT* TT genotypes were significantly associated with increased risk of prostate cancer among men diagnosed younger than 65 years of age. Generally, for early-onset cancer, a strong genetic impact on the origin of the disease has been suggested, and Fulle et al. (28) observed that catalase activity is substantially lower in older than younger individuals. Thus, low CAT activity resulting from genotype could impart an increased risk of prostate cancer among younger individuals, which could be comparable to risk associated with natural declines that accompany aging. However, these findings could be spurious due to multiple testing and small sample size in stratified analyses and require replication in additional studies.

There have been few investigations of potential relationships between *GPX1* genetic polymorphisms and prostate cancer risk, except for one study that assessed the *GPX1* GCG repeat, finding no relationships with prostate cancer risk (29). In our study, we did not find an overall relationship between *GPX1* Pro200Leu polymorphism and prostate cancer. The C allele of *GPX1* has been associated with an increased risk of breast (30), bladder (31), and lung cancer (21), but other studies, including breast, have found no associations (32-35).

Because each variant, alone, may have negligible effects on oxidative burden, we also considered the joint effects of ''high-risk'' alleles for *MnSOD, CAT*, and *GPX1*. Although no dose effect was noted for each additional allele, carriage of five or

six variants was associated with a 2-fold increase in prostate cancer risk, indicating a threshold effect, although the confidence interval included unity. In a similar approach, Sutton et al. (36) found combined effects of *GPX1* and *MnSOD* in reduced risk of hepatocellular carcinoma. Because ultimate levels of ROS are likely dependent on the neutralization by CAT and GPX1 of H_2O_2 produced by MnSOD, it is reasonable to expect that their combined effects may be much more important than single SNPs alone.

As a potential limitation, all CARET participants were heavy smokers or had occupational exposure to asbestos. Data from previous studies indicate that current smokers tend to have higher DNA repair capabilities than former or nonsmokers (37, 38). Thus, results in this high-risk cohort may not be generalizable because of differential response to the chronic exposure. In addition, the controls matched on cases were not necessarily free of other cancers except for prostate and lung cancer. However, there were no significant differences in distributions of genetic polymorphisms and other characteristics between controls with other cancers and those free from cancer (data not shown). The nested case-control design in this prospective cohort is a strength of the study because controls are selected from the same population as the cases, preventing potential bias in standard case-control studies.

In conclusion, these results indicate that genetic polymorphisms in genes relevant to oxidative stress (*MnSOD*, *CAT*, *GPX1*) may not play an important role in the development of prostate cancer, particularly among heavy smokers and men with occupational exposure to asbestos. Furthermore, exogenous exposures that may impact oxidative burden do not seem to modify effects of these genotypes in this population.

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