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Plasma antioxidants, genetic variation in SOD2, CAT, GPX1, GPX4, and prostate cancer survival

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Abstract

Background—Antioxidants may reduce risk of aggressive prostate cancer, and single nucleotide polymorphisms (SNPs) in antioxidant genes may modify this association.

Methods—We used Cox proportional hazards regression to examine circulating prediagnostic alpha-tocopherol, gamma-tocopherol, and lycopene; SNPs in *SOD2* (n=5), *CAT* (n=6), *GPX1* (n=2), *GPX4* (n=3); and their interactions and risk of lethal prostate cancer among 2,439 men with nonmetastatic prostate cancer in the Health Professionals Follow-up Study and Physicians' Health Study.

Results—We observed 223 events over a median follow-up of 10 years. Higher alpha-tocopherol levels were associated with lower risk of lethal prostate cancer (hazard ratio (HR) 3rd v. 1st quartile (Q): 0.51; 95% confidence interval (CI): 0.30, 0.89; HR 4th v. 1st Q: 0.68; 95% CI: 0.41, 1.13; *p*-trend: 0.02). Men homozygous for the less common allele (G) at rs3746165 in *GPX4* had a 35% lower risk of lethal prostate cancer compared to men homozygous for the more common allele (A) (HR: 0.65; 95% CI: 0.43, 0.99). Among men homozygous for the less common allele in rs3746165, high gamma-tocopherol levels were associated with a 3.5-fold increased risk of lethal prostate cancer (95% CI: 1.27, 9.72; *p*-value: 0.02; interaction *p*-value 0.01).

Conclusions—Among men with nonmetastatic prostate cancer, higher circulating prediagnostic alpha-tocopherol may be associated with lower risk of developing lethal disease. Variants in *GPX4* may be associated with risk of lethal prostate cancer, and may modify the relation between gamma-tocopherol and prostate cancer survival.

Corresponding author: Erin Van Blarigan ScD, UCSF, Helen Diller Cancer Research Bldg, 1450 3rd St., San Francisco, CA 94158; (415) 514-4925; erin.vanblarigan@ucsf.edu**Conflicts of interest:** None declared..

Impact—Circulating tocopherol levels and variants in GPX4 may affect prostate cancer progression.

Keywords

tocopherol; lycopene; prostate cancer; survival; genetics

INTRODUCTION

Observational studies and secondary analyses of randomized controlled trials suggest that antioxidants, including alpha-tocopherol, selenium, and lycopene, may reduce risk of prostate cancer, particularly aggressive disease (1-10). In contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) reported that alpha-tocopherol supplementation was associated with an increased risk of overall prostate cancer, and there was no association between selenium supplementation and risk of prostate cancer (11). Prostate cancer is highly heterogeneous in its prognosis however, and risk factors for aggressive disease, including advanced stage and poorly differentiated (e.g. high Gleason sum) tumors, likely differ from risk factors for total incident prostate cancer (12). In addition, several single nucleotide polymorphisms (SNPs) in antioxidant genes have been associated with prostate cancer, and these may modify the relations between circulating or dietary antioxidants and prostate cancer (13, 14). Thus, the discrepant results for antioxidants and prostate cancer may be due to a lack of focus on clinically relevant, advanced forms of the disease or the inability to account for the impact of genetic variants on antioxidant metabolism and availability.

We previously reported in the Health Professionals Follow-up Study that supplemental alpha-tocopherol was associated with a statistically non-significant lower risk of incident metastatic or fatal prostate cancer among current smokers and recent quitters (events=55; hazard ratio (HR) comparing men who consumed 100 IU/d v. none: 0.44; 95% confidence interval (CI): 0.18, 1.07) (15). In addition, we reported in the Physicians Health Study that plasma lycopene was inversely associated with risk of incident aggressive prostate cancer, defined as extraprostatic disease or Gleason sum 7 (odds ratio (OR) comparing the highest v. lowest quintile: 0.56; 95% CI: 0.34, 0.91)(5), and the rs4880 SNP in *SOD2* modified the association between plasma selenium and risk of aggressive prostate cancer (16). Higher levels of plasma selenium were associated with a lower risk of incident aggressive prostate cancer among men with the AA genotype. All of our previous reports examined risk of incident disease (e.g. whether levels of antioxidant nutrients in healthy men were associated with risk of being diagnosed with prostate cancer). Few studies have examined whether circulating antioxidants or germline genetic variants in antioxidant genes are associated with prostate cancer outcomes among men with nonmetastatic prostate cancer. Such analyses may identify factors that affect the growth of prostate tumors, and inform the development of clinical strategies to delay or deter disease progression. In one of the few studies conducted among men with prostate cancer to date, Watters et al. reported that pre-diagnostic circulating alpha-tocopherol levels were associated with a lower risk of prostate cancer death among Finnish smokers with prostate cancer (10).

Thus, we conducted a survival analysis to examine whether pre-diagnostic circulating alpha-tocopherol, gamma-tocopherol, or lycopene levels were associated with lower risk of lethal prostate cancer among men initially diagnosed with non-metastatic prostate cancer in the Health Professionals Follow-up Study and the Physicians' Health Study. In addition, we examined whether SNPs in *SOD2*, *CAT*, *GPX1*, or *GPX4* were associated with risk of progression to lethal prostate cancer, and whether these SNPs modified associations between the circulating antioxidants and risk of lethal prostate cancer. *SOD2* was chosen based on the previous reports (14, 16, 17), and *CAT*, *GPX1*, and *GPX4* were included because they reduce hydrogen peroxide, a byproduct of SOD reactions, to water. We hypothesized that higher circulating alpha-tocopherol and lycopene would be associated with lower risk of lethal prostate cancer; and that the rs4880 SNP in *SOD2* would modify these relations such that the inverse associations would be strongest among men with the AA genotype in rs4880.

MATERIALS and METHODS

Study populations

The Health Professionals Follow-up Study (HPFS) is a prospective cohort initiated in 1986 among 51,529 male health professionals 40-75 years of age. At baseline, the men completed a questionnaire on medical diagnoses, medication use, physical activity, weight, height, and smoking. These data have been updated every two years. Participants also completed a food frequency questionnaire at baseline and every four years thereafter. In 1993-1995, 18,159 men donated blood samples. For this analysis, we utilized covariate data from the 1994 questionnaire to correspond with the time of blood draw.

The Physicians Health Study (PHS) was a randomized controlled trial of aspirin and beta-carotene supplementation initiated in 1982 among 22,071 male physicians. The interventions were stopped in 1988 (aspirin) and 2003 (beta-carotene), and the men have been followed via annual questionnaires on health behaviors and biannual postcards to ascertain disease endpoints. Blood was collected in August 1982 to December 1984 from 14,916 men prior to randomization. Covariate data were obtained from the baseline questionnaire to correspond with the time of blood draw.

In the HPFS and PHS, participants were mailed a blood collection kit, and EDTA-preserved samples were received and processed within 24 hours of blood draw and stored in liquid nitrogen freezers. The genotyping and plasma assessments were originally conducted in nested case-control studies of prostate cancer incidence in the HPFS and PHS, as previously described (5, 16, 18, 19). Incident prostate cancer cases and controls were selected using a risk-set sampling design at three time points in the HPFS (1996, 1998, 2000). In the PHS, incident cases (diagnosed up to 2005) and controls were selected using a risk-set sampling design and additionally matched on age at baseline and smoking status (never, former, current). Prostate cancer cases, and controls who were subsequently diagnosed with prostate cancer during follow-up, were eligible for this analysis.

Prostate cancer follow-up

In the HPFS and PHS, men were asked if they have been diagnosed with prostate cancer every two years, and over 90% of surviving participants responded in each questionnaire cycle. After a report of prostate cancer, we request permission to obtain their medical records to verify the diagnosis and abstract clinical information such as prostate specific antigen (PSA) levels, Gleason sum, and clinical stage. In addition, prostate cancer-specific questionnaires were mailed to participants with prostate cancer to obtain information on their disease course, including events of metastases.

Our main outcome was lethal prostate cancer, defined as distant organ metastases or death due to prostate cancer. Deaths were ascertained via mail, telephone, and review of the National Death Index; follow-up for mortality is at least 98% complete (20). The cause of death and presence of metastases was verified via medical records and death certificates.

Circulating antioxidant nutrient assessments

We assessed circulating alpha-tocopherol, gamma-tocopherol, and lycopene in this study because these nutrients have been previously associated with risk of aggressive prostate cancer (1, 5, 10, 21-23). Blood levels of selenium were not available in the HPFS, and therefore we were unable to examine this nutrient. The methods to assess lycopene in the HPFS (24), and lycopene and tocopherols in PHS (5), have been previously described. In HPFS, alpha- and gamma-tocopherol were quantified from ~ 200 µl or serum with tocol (Matreya, Pleasant Gap, PA) used as an internal standard. Ethanol was used to precipitate serum proteins and hexane was used to extract tocopherols. Hexane extracts were dried in a Speedvac concentrator (Savant model AES 1010, Farmingdale, NY) and stored in a -80 C until analysis (~ 1-2 days). The mobile phase was acetonitrile:methanol:chloroform, 47:47:6. The lab participates in the NIST micronutrient proficiency testing program and values for tocopherols are within ~8% of the medium. The mean coefficient of variations for all nutrients in HPFS and PHS examined were 12% or less.

SNPs, DNA, and genotyping

The antioxidant defense system includes superoxide dismutases (SODs), catalase (CAT), and glutathione peroxidases (GPX). SOD enzymes catalyze the conversion of superoxide radicals into oxygen and hydrogen peroxide, and CAT and GPXs reduce hydrogen peroxide to water. In HPFS and PHS, SNPs that capture common variation (>5%) at a linkage disequilibrium $r^2 > 0.8$ within *SOD2*, *CAT*, *GPX1*, and *GPX4* as well as 5 kb upstream and downstream of the genes were selected using the HapMap database and the Tagger Pairwise program. DNA was extracted from whole blood and genotyping was done with Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight mass spectrometry technology. All SNPs had >91% genotype success rates. The SNPs included in this analysis were: rs4880, rs7855, rs2842980, rs5746151, and rs6917589 in *SOD2*; rs511895, rs769217, rs1001179, rs2076556, rs11032700, and rs11032703 in *CAT*; rs3448 and rs1800668 in *GPX1*; rs2074452, rs3746165, and rs4239605 in *GPX4*.

Inclusion/exclusion criteria

Of the 4,533 men who donated blood in the HPFS and PHS and were subsequently diagnosed with prostate cancer, 2958 men (65%) had data on at least one of the nutrients or SNPs of interest. We excluded men who had metastatic disease at diagnosis or were missing clinical stage (n=375) and non-Caucasian men to limit the potential for population stratification (n=144), leaving 2,439 men for analysis.

Statistical analysis

We used Cox proportional hazards regression to examine circulating alpha-tocopherol, gamma-tocopherol, and lycopene in relation to risk of lethal prostate cancer. In HPFS, person-time was contributed from date of diagnosis until date of lethal prostate cancer, death from another cause, or end of follow-up (January 31, 2010), whichever occurred first. In PHS, person-time was contributed from date of diagnosis until date of lethal prostate cancer, death from another cause, or end of follow-up (October 2, 2008 or date of the last available questionnaire if returned after October 2, 2008), whichever occurred first. We also conducted sensitivity analyses examining the plasma nutrients and SNPs in relation to prostate cancer-specific mortality (vs. the combined endpoint of distant metastases and prostate cancer death). For these sensitivity analyses, person-time was contributed from prostate cancer diagnosis until death or end of follow-up.

We categorized the antioxidants into batch-specific quartiles (e.g. within each batch, we categorized all study participants into quartiles and assigned each man a value of 0,1,2, or 3 corresponding to his quartile of the circulating nutrient of interest) and modeled the resulting ordinal variable using indicator variables. To test for evidence of a linear trend, we modeled the quartile score as a continuous term (0, 1, 2, 3). Model 1 was adjusted for age at diagnosis (continuous), circulating cholesterol (batch-specific quartiles), cohort (HPFS, PHS), and time from blood draw to diagnosis (continuous). Blood cholesterol was included because tocopherols and lycopene are transported in the blood via lipoproteins (25). Model 2 was additionally adjusted for body mass index (BMI; <25, 25-29.9, 30 kg/m^2) and smoking status (never, quit 10 y, quit <10 y, current). To examine whether the relations between pre-diagnostic circulating antioxidants and lethal prostate cancer were independent of known clinical prognostic factors, we examined a model adjusting for PSA levels at diagnosis (tertiles plus an indicator for missing), Gleason sum (<7, 7, >7, missing), and clinical T-stage (T1/T2 v. T3). Based on the results of the Alpha-tocopherol Beta-Carotene trial among male Finnish smokers (4, 10), we performed a sensitivity analysis to examine whether smoking status at the time of blood draw modified the association between alpha-tocopherol and risk of lethal prostate cancer by including a cross-product term between alpha-tocopherol levels (continuous) and dichotomized smoking status (never or quit 10 y v. quit <10 y or current), and using a wald test to examine whether there was evidence of an interaction. Too few men were current smokers at the time of blood draw (4%) to examine this category separately from recent quitters. Additionally, due to the difference in length of time from blood draw to diagnosis in the HPFS and PHS, we performed a sensitivity analysis stratified by cohort.

Using Pearson's goodness-of-fit test, one SNP, rs554518 in *CAT*, violated Hardy-Weinberg equilibrium (*p*-value: 0.03); therefore we excluded this SNP from further analyses. We used Cox proportional hazards regression to examine the relations between genetic variants in *SOD2*, *CAT*, *GPX1*, and *GPX4* and time to lethal prostate cancer, adjusting for age at diagnosis. Person-time was defined as stated above. SNPs were analyzed under additive and co-dominant genetic models. If the rare homozygous genotype was present in <5% of the study population, we analyzed the SNP using a dominant genetic model. In addition, we used SAS PROC HAPLOTYPE to generate haplotype scores conditional on the observed genotypes; haplotypes with a frequency <0.05 were combined. We modeled the haplotype probabilities as continuous covariates in Cox proportional hazards models adjusting for age at diagnosis. We assumed an additive mode of inheritance and used the most common haplotype within the gene of interest as the reference (26).

In addition, we examined whether the SNPs modified the relations between the circulating antioxidants and risk of lethal prostate cancer. We created cross-product terms between the genetic variants assuming an additive genetic model and the plasma nutrient levels dichotomized at the batch-specific median, and used a Wald test to test for evidence of effect modification. Lastly, we examined whether any of the SNPs were associated with circulating levels of alpha-tocopherol, gamma-tocopherol, or lycopene under an additive genetic model using multivariate linear regression adjusting for age at diagnosis and circulating cholesterol. The circulating nutrient levels were log-transformed to improve normality for the linear regression models.

The Institutional Review Boards of the Harvard School of Public Health and University of California, San Francisco approved this study. All analyses were performed using SAS version 9.2 and *P* values were two-sided.

RESULTS

We observed 223 events of lethal prostate cancer (84 in the HPFS, 139 in the PHS) during a median follow-up of 10 years (9 years in the HPFS, 12 years in the PHS) among 2,439 men initially diagnosed with non-metastatic prostate cancer. Characteristics of our study population are described in **Table 1**. Overall, the men from HPFS and PHS were similar, with a mean age at diagnosis of 70 years, PSA at diagnosis of 7 ng/ml, over 90% diagnosed with clinical T-stage T2 or less, and over 50% had Gleason sum 2-6. The median time from blood draw to diagnosis was 8 years (interquartile range (IQR): 5, 13), 6 years in the HPFS and 13 years in the PHS. In addition, the distribution of the genetic variants was similar between the HPFS and PHS cohorts (**Supplementary Table 1**); therefore the main analyses were conducted in the combined study population.

Higher circulating pre-diagnostic levels of alpha-tocopherol were associated with a lower risk of lethal prostate cancer (*p*-trend: 0.02) (**Table 2**). Men in the third quartile of plasma alpha-tocopherol had a 49% lower risk of lethal prostate cancer compared to men in the first quartile (HR: 0.51; 95% CI: 0.30, 0.89), and men in the fourth quartile had a statistically non-significant 32% lower risk of lethal prostate cancer compared to men in the first quartile (HR: 0.68; 95% CI: 0.41, 1.13). The relation was unchanged when adjusting for clinical T-

stage, Gleason sum, and PSA at diagnosis, and became stronger when examining risk of prostate cancer-specific mortality (HR Q3 vs. Q1: 0.43; 95% CI: 0.24, 0.78; HR Q4 vs. Q1: 0.55; 95% CI: 0.32, 0.96; *p*-trend: 0.003). Smoking status at blood draw did not appear to modify the relation between alpha-tocopherol and risk of lethal prostate cancer, but we had limited power to examine this interaction due to few current smokers or recent quitters in our study population. Furthermore, the inverse relation was apparent in both cohorts, although stronger and more consistent in the HPFS (HR Q3 v. Q1: 0.45; 95% CI: 0.21, 0.99; HR Q4 v. Q1: 0.47; 95% CI: 0.22, 1.00; *p*-trend: 0.02) compared to the PHS (HR Q3 v. Q1: 0.57; 95% CI: 0.27, 1.23; HR Q4 v. Q1: 0.85; 95% CI: 0.43, 1.70; *p*-trend: 0.23), possibly due to the shorter time between blood draw and diagnosis in the HPFS. There were no associations between pre-diagnostic circulating gamma-tocopherol or lycopene and risk of lethal prostate cancer. Circulating levels of alpha-tocopherol and gamma-tocopherol were not correlated in our study population (r=-0.04; *p*-value: 0.12), and the results were similar when mutually adjusting alpha-tocopherol and gamma-tocopherol (HR Q3 v. Q1: 0.53; 95% CI: 0.31, 0.92; HR Q4 v. Q1: 0.74; 95% CI: 0.44, 1.25; *p*-trend: 0.05).

Men who were homozygous for the less common G allele in rs3746165, a SNP in *GPX4*, had a 35% decreased risk of lethal prostate cancer compared to men who were homozygous for the more common A allele (HR: 0.65; 95% CI: 0.43, 0.99) (**Table 3**). In addition, men who were heterozygous at the rs6917589 SNP in *SOD2* had a 37% increased risk of lethal prostate cancer compared to men homozygous for the more common A allele (HR: 1.37; 95% CI: 1.02, 1.83); however there was no increased risk among men homozygous for the less common G allele (HR: 1.17; 95% CI: 0.63, 2.19) nor was there statistically significant evidence of a linear trend in the additive model (HR: 1.22; 95% CI: 0.97, 1.52; *p*-value: 0.09). Additionally, two haplotypes, one in GPX4 and one in CAT, were associated with risk of lethal prostate cancer (**Supplementary Table 2**). Given the number of tests conducted, these results may be due to chance and should be interpreted cautiously.

In addition, two SNPs in *GPX4*, including rs3746165, appeared to modify the relation between circulating gamma-tocopherol and lethal prostate cancer (**Table 4**). Among men who were homozygous for the less common allele in rs3746165, men who had circulating gamma-tocopherol levels at or above the batch-specific median had a 3.5-fold higher risk of lethal prostate cancer compared to men below the median (HR: 3.52; 95% CI: 1.27, 9.72; *p*-value: 0.02). There was no association between circulating gamma-tocopherol and risk of lethal prostate cancer among men who were heterozygous or homozygous for the more common allele (*p*-interaction: 0.01). Similarly, among men who were homozygous for the less common allele in rs4239605, men who had circulating gamma-tocopherol levels at or above the batch-specific median had a 6.4-fold increased risk of lethal prostate cancer (HR: 6.35; 95% CI: 1.78, 22.74; *p*-trend: 0.005). There was no association between circulating gamma-tocopherol and risk of lethal prostate cancer among men who were heterozygous or homozygous for the more common allele (*p*-interaction: 0.003). All other interactions were evaluated and are not shown; only interactions with p<0.05 are summarized in Table 4.

Lastly, three SNPs, rs511895 and rs1001179 in *CAT* and rs3746165 in *GPX4*, were associated with circulating levels of alpha-tocopherol (rs511895 β: -0.04; standard error (se): 0.02; *p*-value: 0.02; rs1001179 β: -0.04; standard error (se): 0.02; *p*-value: 0.03; rs3746165

β: -0.03; standard error (se): 0.02; *p*-value: 0.04). We examined the relation between rs3746165 and risk of lethal prostate cancer adjusting for circulating alpha-tocopherol levels to examine if the relation between this SNP and lethal prostate cancer may be mediated through alpha-tocopherol levels. In that model, the estimates for the relation between both the SNP and alpha-tocopherol levels and risk of lethal prostate cancer were essentially unchanged, although no longer statistically significant (rs3746165 AA/AG (ref.) v. GG HR: 0.65; 95% CI: 0.40, 1.04; *p*-value: 0.07; alpha-tocopherol HR Q3 v. Q1: 0.57; 95% CI 0.32, 1.00; HR Q4 v. Q1: 0.65; 95% CI: 0.37, 1.14; *p*-trend: 0.02). The lack of statistical significance may be due to the correlation between the SNP and plasma alpha-tocopherol levels (27). None of the SNPs were associated with circulating levels of gamma-tocopherol or lycopene.

DISCUSSION

In this prospective survival analysis among men initially diagnosed with non-metastatic prostate cancer, we observed an inverse association between circulating alpha-tocopherol prior to diagnosis and risk of lethal prostate cancer. Circulating gamma-tocopherol and lycopene were not associated with progression to lethal prostate cancer. In addition, one SNP (rs3746165) in *GPX4* was associated with risk of lethal prostate cancer, and this SNP and another in the same gene appeared to modify the relation between circulating gamma-tocopherol and risk of lethal prostate cancer.

Alpha-tocopherol has been extensively studied in relation to incident prostate cancer, with inconsistent results. Secondary analyses of the Alpha-Tocopherol, Beta-Carotene trial indicated that male smokers randomized to 50 mg/d of alpha-tocopherol for a median of six years had a 32% lower risk of prostate cancer (95% CI: -47% to -12%) and a 41% lower risk of dying from prostate cancer (95% CI: -65% to -1%) compared to placebo (4). A prospective analysis among the 1891 men diagnosed with prostate cancer during the trial reported that men in the highest quintile of serum alpha-tocopherol at baseline had lower risk of prostate cancer-specific death compared to men in the lowest quintile (10). Several, although not all (1, 15, 28, 29), prospective cohort studies have also reported an inverse association between dietary or circulating vitamin E and risk of aggressive prostate cancer (22, 23), particularly among smokers. In contrast, SELECT reported an increased risk of prostate cancer among men assigned to 400 IU/d of alpha-tocopherol (11); however, the study population in SELECT was replete with vitamin E at baseline and there were too few advanced prostate cancers to examine this outcome.

Our results are consistent with those from the Alpha-tocopherol Beta-Carotene trial of Finnish male smokers, and suggest that higher circulating levels of alpha-tocopherol prior to diagnosis may be associated with a lower risk of progression to lethal prostate cancer (10). Further, only 14% of the men in our study population were current smokers or had quit smoking within 10 years of blood donation, and thus the beneficial effects of alpha-tocopherol may not be restricted to smokers. Our observations are consistent with two potential scenarios. First, higher alpha-tocopherol levels may predispose healthy men to develop a form of prostate cancer that has less potential for progression. Second, if the alpha- tocopherol levels assessed in our study are correlated with levels after diagnosis, it is

possible that higher alpha-tocopherol levels after diagnosis of non-metastatic prostate cancer may deter or delay progression of the disease. A second assessment of alpha-tocopherol levels obtained after diagnosis of nonmetastatic prostate cancer is needed to differentiate these two potential scenarios, and would be of interest in future studies.

In this survival analysis among men diagnosed with non-metastatic prostate cancer, we did not observe an association between pre-diagnostic circulating levels of lycopene and risk of lethal prostate cancer. We previously reported in a nested case-control study in the PHS that men in the fifth quintile of plasma lycopene had a 44% lower risk of incident aggressive prostate cancer, defined as extraprostatic disease or Gleason sum 7 tumors (n=259), compared to men in the lowest quintile (5). Together, these data suggest that lycopene may act early in the disease process, affecting the initiation of aggressive prostate cancer rather than its progression. Additional studies with long follow-up and multiple assessments of circulating lycopene would be of interest to identify the time window during which lycopene may act in the natural history of aggressive prostate cancer.

One SNP in *GPX4*, rs3746165, were associated with risk of lethal prostate cancer, and modified the relation between circulating gamma-tocopherol and risk of lethal prostate cancer. This SNP is located 2 kb upstream of the gene, and has not been previously reported to be associated with risk of prostate cancer or prostate cancer survival; thus, the results observed might be due to chance. Another SNP in *GPX4* (rs2074452) was associated with prostate cancer-specific mortality in a study with 81 events and a median follow-up of 9 years, but this SNP was not associated with lethal prostate cancer in our population (30). In addition, we did not observe an association between rs4880, a SNP in *SOD2*, and risk of lethal prostate cancer. rs4880 has been associated with risk of prostate cancer in multiple study populations (14), including risk of aggressive disease among men initially diagnosed with non-metastatic prostate cancer (31). Overall, studies on germline genetic variants and prostate cancer survival are limited, and studies in larger populations are needed.

This study had several limitations. First, we had only one assessment of the circulating antioxidant nutrients taken a median of eight years prior to diagnosis. Among 144 men in the HPFS, two measures of plasma lycopene assessed three to four years apart had an spearman correlation of 0.58 (24). In a different cohort of 166 men, the correlation between serum measures taken 15 years apart was 0.35 for lycopene, 0.61 for alpha-tocopherol, and 0.48 for gamma-tocopherol (32). Thus, while not free from non-differential misclassification, the quartile ranking of plasma levels assessed in this study are likely a fair representation of the quartile ranking at the time of diagnosis, particularly for alpha-tocopherol. Second, we performed many statistical tests, and the statistically significant results that we observed could be due to chance. However, as one of the first studies to examine genetic variants in antioxidants SNPs and risk of lethal prostate cancer, we took an exploratory approach and did not adjust for multiple testing. Independent studies are necessary to replicate the suggestive findings reported here. Third, we examined the combined endpoint of distant metastases or death due to prostate cancer because essentially all fatal cases of prostate cancer are preceded by metastases to distant organs. However, it is possible that the role of antioxidants may differ for metastasis and prostate cancer death; we observed that the relation between plasma alpha-tocopherol and prostate cancer-specific

mortality was stronger than its relation with the combined endpoint that included metastases. Fourth, we combined data from two prospective U.S. cohorts in order to examine the rare outcome of lethal prostate cancer. The time between blood draw and diagnosis of prostate cancer was shorter in the HPFS compared to the PHS, and thus the degree of measurement error for the plasma nutrient levels may differ between the cohorts. We included an indicator variable for cohort in all of our models examining plasma nutrient levels, and analyses stratified by cohort showed that the relation between plasma alpha-tocopherol and risk of lethal prostate cancer was inverse in both populations. Lastly, we restricted our study population to Caucasians to reduce the potential for population stratification; our results may not be generalizable to populations with other racial, and therefore genotype, distributions.

In conclusion, among men initially diagnosed with non-metastatic prostate cancer, higher circulating levels of alpha-tocopherol prior to diagnosis may be associated with a lower risk of developing lethal disease. In addition, genetic variants in *GPX4* may be associated with risk of lethal prostate cancer, and may modify the relation between circulating gamma-tocopherol and risk of lethal prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of 2439 men initially diagnosed with non-metastatic prostate cancer in the Health Professionals Follow-up Study (HPFS) and the Physicians Health Study (PHS).

	HPFS	PHS	Total
No. of participants	1206	1233	2439
Date of diagnosis, median (IQR)	1999 (1997, 2002)	1995 (1991, 1999)	1998 (1994, 2001)
Age at diagnosis, y, mean ± SD	70 ± 7	70 ± 7	70 ± 7
Smoking status at blood draw, %			
Never	50	48	49
Former, quit 10 y	38	36	37
Former, quit <10 y	8	11	10
Current	3	4	4
Missing	1	0	0
Body mass index at blood draw, %			
<25 kg/m^2	50	59	55
25-29.9 kg/m^2	41	39	40
30 kg/m^2	8	3	5
Gleason sum at diagnosis, %			
<7	52	59	56
7	35	23	29
8	11	11	11
Missing	2	7	5
Clinical stage T3, %	2	6	4
PSA at diagnosis, ng/ml, median (IQR)	7 (5, 10)	7 (5, 12)	7 (5, 11)
Alpha-tocopherol, mg/L, median (IQR)	12.3 (9.0, 16.5)	11.1 (9.2, 13.7)	11.4 (9.1, 14.6)
Gamma-tocopherol, mg/L, median (IQR)	1.5 (0.9, 2.3)	1.9 (1.4, 2.5)	1.8 (1.2, 2.4)
Lycopene, ng/ml, median (IQR)	396 (291, 539)	313 (210, 474)	352 (239, 507)
Time from blood draw to diagnosis, y, median (IQR)	6 (3, 8)	13 (9, 17)	8 (5, 13)
Metastases or deaths due to prostate cancer, N (%)	84 (7)	139 (11)	223 (9)

Table 2

Circulating tocopherols and lycopene prior to diagnosis and risk of lethal prostate cancer among men initially diagnosed with non-metastatic prostate cancer in the Health Professionals Follow-up Study and Physicians Health Study.

	Batch-specific quartiles				
	Q1	Q2	Q3	Q4	p-trend[1]
Alpha-tocopherol					
Median alpha-tocopherol, mg/L	7.8	10.2	12.6	18.1	
Events / person-years	36 / 2752	44 / 2810	21 / 2895	29 / 2761	
Model 1 HR (95% CI)[2]	1.0 (ref.)	1.16 (0.74, 1.81)	0.52 (0.30, 0.89)	0.67 (0.40, 1.13)	0.02
Model 2 HR (95% CI)[3]	1.0 (ref.)	1.16 (0.74, 1.81)	0.51 (0.30, 0.89)	0.68 (0.41, 1.13)	0.02
Model 3 HR (95% CI)[4]	1.0 (ref.)	1.08 (0.69, 1.70)	0.50 (0.29, 0.88)	0.68 (0.40, 1.14)	0.02
Gamma-tocopherol					
Median gamma-tocopherol, mg/L	0.8	1.5	2.0	3.0	
Events / person-years	25 / 2664	32 / 2812	43 / 2839	30 / 2878	
Model 1 HR (95% CI)[2]	1.0 (ref.)	1.25 (0.74, 2.11)	1.75 (1.07, 2.88)	1.15 (0.67, 1.98)	0.37
Model 2 HR (95% CI)[3]	1.0 (ref.)	1.26 (0.74, 2.12)	1.74 (1.06, 2.87)	1.09 (0.63, 1.88)	0.49
Model 3 HR (95% CI)[3]	1.0 (ref.)	1.24 (0.73, 2.10)	1.53 (0.93, 2.52)	0.99 (0.56, 1.72)	0.84
Lycopene					
Median lycopene, ng/ml	200	328	451	626	
Events / person-years	31 / 2690	37 / 2870	32 / 2847	32 / 2944	
Model 1 HR (95% CI)[2]	1.0 (ref.)	1.07 (0.66, 1.73)	0.98 (0.60, 1.62)	1.00 (0.60, 1.66)	0.91
Model 2 HR (95% CI)[3]	1.0 (ref.)	1.06 (0.65, 1.71)	0.99 (0.60, 1.63)	1.01 (0.61, 1.69)	0.97
Model 3 HR (95% CI)[3]	1.0 (ref.)	1.17 (0.72, 1.91)	1.23 (0.74, 2.05)	1.21 (0.72, 2.03)	0.46

[1] p-trend calculated by modeling the quartile ordinal score (0, 1, 2, 3) as a continuous variable.

[2] Adjusted for age at diagnosis (continuous), circulating cholesterol (batch-specific quartiles), cohort (HPFS v. PHS), and time between blood draw and diagnosis (continuous).

[3] Adjusted for above variables plus baseline body mass index (BMI; <25, 25-29.9, $\geq$ 30 kg/m^2) and smoking status (never, quit 10+ y, quit <10 y, current).

[4] Adjusted for above variables plus Gleason sum (2-6, 7, 8-10, missing), prostate specific antigen (PSA) at diagnosis (tertiles and an indicator for missing), and clinical T-stage (T1/T2 v. T3).

Table 3

SNPs in *SOD2, CAT, GPX1,* and *GPX4* and risk of lethal prostate cancer among men initially diagnosed with non-metastatic prostate cancer in the Physicians Health Study and the Health Professionals Follow-up Study.

SNP	Genotype Frequencies		Co-dominant Model[1]	Additive Model[1]	
	Deaths/metastases n (%)	All other cases n (%)	HR (95% CI)	HR (95% CI)	*p*-value
SOD2					
rs4880[2]				0.89 (0.73, 1.08)	0.23
TT	58 (28)	507 (24)	1.0 (ref.)		
CT	104 (50)	1031 (50)	0.89 (0.65, 1.23)		
CC	45 (22)	539 (26)	0.79 (0.53, 1.16)		
rs7855[3]				0.70 (0.42, 1.17)	0.17
TT	192 (92)	1886 (90)	1.0 (ref.)		
CT	16 (8)	209 (10)	0.70 (0.42, 1.17)		
CC	0 (0)	5 (0)			
rs2842980				1.05 (0.83, 1.33)	0.67
AA	127 (62)	1310 (63)	1.0 (ref.)		
AT	68 (33)	660 (32)	1.02 (0.76, 1.37)		
TT	11 (5)	98 (5)	1.18 (0.64, 2.19)		
rs5746151[3]				0.76 (0.48, 1.21)	0.25
GG	182 (90)	1830 (88)	1.0 (ref.)		
AG	20 (10)	247 (12)	0.76 (0.48, 1.21)		
AA	0 (0)	8 (0)			
rs6917589				1.22 (0.97, 1.52)	0.09
AA	103 (53)	1255 (61)	1.0 (ref.)		
AG	80 (41)	693 (34)	1.37 (1.02, 1.83)		
GG	11 (6)	109 (5)	1.17 (0.63, 2.19)		
CAT					
rs511895				0.88 (0.72, 1.08)	0.23

SNP	Genotype Frequencies		Co-dominant Model[1]	Additive Model[1]	
	Deaths/metastases n (%)	All other cases n (%)	HR (95% CI)	HR (95% CI)	p-value
rs769217				1.08 (0.86, 1.36)	0.52
CC	81 (40)	728 (35)	1.0 (ref.)		
CT	90 (45)	997 (48)	0.82 (0.61, 1.11)		
TT	31 (15)	333 (16)	0.82 (0.54, 1.24)		
rs1001179				0.97 (0.77, 1.21)	0.76
CC	126 (61)	1263 (60)	1.0 (ref.)		
CT	68 (33)	733 (35)	0.97 (0.72, 1.31)		
TT	14 (7)	94 (5)	1.43 (0.82, 2.48)		
rs2076556[3]				1.08 (0.79, 1.48)	0.64
CC	119 (59)	1222 (59)	1.0 (ref.)		
CT	76 (37)	727 (35)	1.10 (0.83, 1.47)		
TT	8 (4)	117 (6)	0.66 (0.32, 1.35)		
rs11032700				1.08 (0.88, 1.32)	0.48
AA	155 (75)	1566 (76)	1.0 (ref.)		
AG	49 (24)	469 (23)	1.08 (0.79, 1.48)		
GG	2 (1)	25 (1)			
rs11032703[3]				0.88 (0.63, 1.24)	0.47
AA	87 (44)	940 (46)	1.0 (ref.)		
AC	88 (44)	882 (43)	1.11 (0.82, 1.49)		
CC	24 (12)	229 (11)	1.13 (0.72, 1.78)		
CC	164 (80)	1615 (78)	1.0 (ref.)		
CT	36 (18)	415 (20)	0.88 (0.63, 1.24)		
TT	5 (2)	35 (2)			
GPX1					
rs3448				1.10 (0.89, 1.37)	0.39
GG	115 (56)	1178 (57)	1.0 (ref.)		
GA	76 (37)	748 (36)	1.08 (0.81, 1.44)		

SNP	Genotype Frequencies		Co-dominant Model[1]	Additive Model[1]	
	Deaths/metastases n (%)	All other cases n (%)	HR (95% CI)	HR (95% CI)	p-value
AA	15 (7)	126 (6)	1.26 (0.74, 2.16)		
rs1800668				0.97 (0.79, 1.19)	0.74
CC	90 (45)	933 (45)	1.0 (ref.)		
CT	89 (44)	893 (43)	0.98 (0.73, 1.31)		
TT	23 (11)	244 (12)	0.92 (0.58, 1.46)		
GPX4					
rs2074452				1.06 (0.85, 1.31)	0.63
CC	105 (52)	1169 (57)	1.0 (ref.)		
CT	86 (43)	759 (37)	1.22 (0.92, 1.63)		
TT	11 (5)	140 (7)	0.83 (0.45, 1.54)		
rs3746165				0.83 (0.68, 1.01)	0.06
AA	56 (28)	549 (27)	1.0 (ref.)		
AG	110 (55)	990 (49)	1.06 (0.77, 1.46)		
GG	35 (17)	501 (25)	0.65 (0.43, 0.99)		
rs4239605				0.85 (0.69, 1.05)	0.14
AA	52 (30)	498 (26)	1.0 (ref.)		
AG	93 (53)	1006 (52)	0.92 (0.65, 1.29)		
GG	31 (18)	443 (23)	0.71 (0.45, 1.10)		

[1] Adjusted for age at diagnosis (years).

[2] C = alanine (A) and T = valine (V).

[3] The heterozygote and homozygous less common allele categories were combined when <5% of the study population was homozygous for the less common allele.

Table 4

Circulating gamma-tocopherol prior to diagnosis and risk of lethal prostate cancer by two single nucleotide polymorphisms in *GPX4* among men initially diagnosed with non-metastatic prostate cancer in the Health Professionals Follow-up Study and Physicians Health Study.

SNP alleles	Events / person-years	Below batch-specific median HR (95% CI)[1]	Batch-specific median or higher HR (95% CI)[1]	p-value	Interaction p-value
rs3746165					0.01
AA	30 / 2731	1.0 (ref.)	0.86 (0.41, 1.81)	0.69	
AG	63 / 4850	1.0 (ref.)	1.07 (0.64, 1.79)	0.80	
GG	21 / 2624	1.0 (ref.)	3.52 (1.27, 9.72)	0.02	
rs4239605					0.003
AA	28 / 2793	1.0 (ref.)	0.66 (0.30, 1.46)	0.31	
AG	45 / 4666	1.0 (ref.)	1.12 (0.61, 2.04)	0.71	
GG	20 / 2032	1.0 (ref.)	6.35 (1.78, 22.74)	0.005	

2 Interaction p-value calculated by adding a cross-product term between the dichotomized gamma-tocopherol levels (at or above batch-specific median v. below) and genotype (additive model) in a model that included the genotype, dichotomized gamma-tocopherol levels, circulating cholesterol (batch-specific quartiles), cohort (HPFS v. PHS), and time between blood draw and diagnosis (continuous).

[1] Adjusted for age at diagnosis (continuous), cohort (HPFS v. PHS), circulating cholesterol levels (batch-specific quartiles), and time between blood draw and diagnosis (continuous).