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## Antioxidant and Vitamin E Transport Genes and Risk of High-Grade Prostate Cancer and Prostate Cancer Recurrence

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### Abstract

**BACKGROUND**—Observational studies suggest an inverse association between vitamin E and risk of prostate cancer, particularly aggressive tumors. However, three large randomized controlled trials have reported conflicting results. Thus, we examined circulating vitamin E and vitamin E-related genes in relation to risk of high-grade prostate cancer and prostate cancer recurrence among men initially diagnosed with clinically organ-confined disease.

**METHODS**—We measured circulating  $\alpha$ - and  $\gamma$ -tocopherol and genotyped 30 SNPs in *SOD1*, *SOD2*, *SOD3*, *TTPA*, and *SEC14L2* among 573 men with organ-confined prostate cancer who underwent radical prostatectomy. We examined associations between circulating vitamin E, genotypes, and risk of high-grade prostate cancer (Gleason grade 8 or 7 with primary score 4;  $n = 117$ ) using logistic regression, and risk of recurrence (56 events; 3.7 years median follow-up) using Cox proportional hazards regression.

**RESULTS**—Circulating  $\gamma$ -tocopherol was associated with an increased risk of high-grade prostate cancer (Q4 v. Q1 odds ratio [OR] = 1.87; 95% confidence intervals [CI]: 0.97–3.58;  $P_{\text{trend}} = 0.02$ ). The less common allele in *SOD3* rs699473 was associated with an increased risk of high-

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grade disease (T > C: OR = 1.40, 95% CI: 1.04–1.89). Two independent SNPs in *SOD1* were inversely associated with prostate cancer recurrence in additive models (rs17884057 hazard ratio [HR] = 0.49, 95% CI: 0.25–0.96; rs9967983 HR = 0.62, 95% CI: 0.40–0.95).

**CONCLUSIONS**—Among men with clinically organ-confined prostate cancer, genetic variation in *SOD* may be associated with risk of high-grade disease at diagnosis and disease recurrence. Circulating  $\gamma$ -tocopherol levels may also be associated with an increased risk of high-grade disease at diagnosis.

### Keywords

prostate cancer; vitamin E; genetic polymorphisms; Gleason grade; recurrence

## INTRODUCTION

Prostate cancer is the most common and second most deadly non-skin cancer among men in the United States (US) [1]. However, many more men are diagnosed with prostate cancer, 241,740 in 2012, than will die from the disease, 28,170 in 2012, due to the vast heterogeneity of prostate cancer tumors [1]. Identifying risk factors for aggressive prostate cancer and prostate cancer recurrence could shed light on the biology of lethal prostate cancer.

Several prospective studies have reported an inverse association between vitamin E, a potent antioxidant, and risk of incident prostate cancer, particularly for aggressive disease [2–4]. However, large randomized controlled trials of vitamin E supplementation and prostate cancer have reported conflicting results. In the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, a randomized controlled trial of 29,133 Finnish male smokers, supplementation with 50 mg of  $\alpha$ -tocopherol per day was associated with a 32% and 41% lower risk of prostate cancer incidence and mortality, respectively [5]. Conversely, the Physician's Health Study II observed no difference in prostate cancer incidence or mortality among US men randomized to 400 IU vitamin E every other day compared to placebo [6]. Furthermore, in 2008, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) in the US was terminated early when an interim analysis demonstrated no benefit with selenium or vitamin E supplementation, as well as a trend towards increased risk of prostate cancer with vitamin E supplementation alone [7]. A recent updated analysis with additional follow-up confirmed the increased risk of incident prostate cancer with vitamin E supplementation in SELECT [8].

The relation between vitamin E and prostate cancer risk may be influenced by genetic variation in antioxidant and vitamin E-related genes, which could partially account for the conflicting results of the randomized controlled trials. Superoxide dismutase (*SOD*) is a family of enzymes found in the cytoplasm (*SOD1*), mitochondria (*SOD2*), and extracellular space (*SOD3*) that detoxify superoxide free radicals and protect cells from oxidative stress and toxicity. Experimental studies indicate that *SOD2* may function as a tumor suppressor gene, potentially through apoptotic and anti-proliferative mechanisms [9]. Expression of *SOD1* and *SOD2* is decreased in prostatic intraepithelial neoplasia and prostate carcinoma compared to benign epithelium [10]. In a nested case-control study within the Prostate,

Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, carriers of the Ala variant in the *SOD2* gene were at increased risk of prostate cancer; however, participants in the highest quartile of vitamin E intake were somewhat protected ( $P_{\text{interaction}} = 0.06$ ) [11]. An interaction was also observed between circulating  $\alpha$ -tocopherol and *SOD2* rs4880; the inverse association between circulating  $\alpha$ -tocopherol and risk of prostate cancer was stronger among men with the AA genotype compared to men with the V allele ( $P_{\text{interaction}} = 0.03$ ) [12].

Other genetic variants may further modify the relation between circulating vitamin E and prostate cancer through effects on intracellular transport of vitamin E isoforms. Proteins encoded by *SEC14L2* facilitate transport of  $\alpha$ -tocopherol into the nucleus and other organelles [13]. *TTPA* encodes  $\alpha$ -tocopherol transport protein ( $\alpha$ -TTA), a vitamin E transport protein that incorporates  $\alpha$ -tocopherol preferentially into very low density lipoproteins (VLDL) [14]. In the ATBC study, significant interactions were observed between two variants in *SEC14L2* (rs2299825 and rs2299829) and vitamin E supplementation in relation to the risk of prostate cancer; men who were homozygous for either common allele experienced a reduced risk of prostate cancer with vitamin E supplementation (rs2299825 odds ratio [OR] = 0.52, 95% confidence interval [CI]: 0.30, 0.90; rs2299829 OR = 0.64, 95% CI: 0.46, 0.88), whereas a non-significant increased risk of prostate cancer was observed among carriers of either variant allele (both  $P_{\text{interaction}} < 0.05$ ) [15]. Genetic variation in *TTPA* appears to be associated with serum vitamin E levels, but has not been associated with prostate cancer risk [15].

No previous study has examined circulating tocopherols and genetic variation in vitamin-E related genes in relation to aggressive prostate cancer using a case-only design. This study design addresses the question of whether vitamin E and vitamin-E related genes play a role in the progression of localized prostate cancer to aggressive disease. Thus, we evaluated the association between single nucleotide polymorphisms (SNPs) in *SOD1*, *SOD2*, *SOD3*, *SEC14L2*, and *TTPA* and risk of high-grade prostate cancer and prostate cancer recurrence among 573 men initially diagnosed with organ-confined prostate cancer who underwent radical prostatectomy as primary treatment at the University of California, San Francisco (UCSF). These genes were selected because they have been previously reported to modify the relation between vitamin E and prostate cancer or modify the relation between vitamin E intake and circulating tocopherol levels (*TTPA*).

## METHODS

### Study Population

This study was conducted among men initially diagnosed with clinically organ-confined prostate cancer between 2000 and 2007 who underwent radical prostatectomy as primary treatment at UCSF, consented, and provided fasting blood samples and residual tissue for research to our tissue core (n = 1,134). We excluded men who had neoadjuvant treatment (e.g., hormones), men who did not consent to clinical follow-up, and men with missing clinical data (e.g., pre-surgical PSA, Gleason score, stage), leaving 1,003 eligible for analyses. Due to budgetary restrictions, we selected 700 of the 1,003 men who met these criteria. We preferentially selected men with high Gleason grade prostate cancer (Gleason

sum 8 or 7 with major Gleason score 4) to enable us to identify risk factors for aggressive disease; 32% of the men in our study population had high-risk disease versus 22% of all men who underwent radical prostatectomy at UCSF between 2000 and 2007. Of the 700 patients selected for this study, 573 had sufficient DNA and plasma available for analysis. The median time from diagnosis to radical prostatectomy/date of blood draw was 3.6 months and the median time from diagnosis to disease recurrence was 3.7 years. This study was approved by the Institutional Review Board of the University of California, San Francisco.

### Circulating Vitamin E measurement

Fasting plasma samples were obtained just prior to radical prostatectomy, and  $\alpha$ - and  $\gamma$ -tocopherol concentrations were assessed using high performance liquid chromatography (HPLC) at the Fred Hutchinson Cancer Research Center (Seattle, WA). A hexane extract of plasma was injected onto a C-18 Spherisorb ODS-2 HPLC column (3  $\mu$ m, 3.0 mm  $\times$  125 mm, Waters PSS838528) and eluted with an isocratic solvent consisting of 76% acetonitrile, 12% tetrahydrofuran, 5% methanol, 7% water, 0.025% ammonium acetate and 0.05% diethyl amine (v/v) at a flow rate of 0.7 ml/min.  $\alpha$ - and  $\gamma$ -tocopherols were detected at 292 nm. Standard curves were generated with commercially available pure chemicals and  $\alpha$ -tocopherol acetate was used as an internal standard. The HPLC was a fully automated Agilent 1100 LC system equipped with quaternary pump, electronic degasser, thermostated column compartment (set at 25°C), automatic sampler, diode array detector and Chem-Station software. A pooled plasma quality control sample was run with each batch of study samples to monitor assay performance. The coefficient of variation was 1.4% for both  $\alpha$ -tocopherol and  $\gamma$ -tocopherol. Circulating  $\alpha$ -tocopherol and  $\gamma$ -tocopherol levels were inversely correlated ( $r = -0.10$ ,  $P = 0.015$ ).

### Genomic DNA and Genotyping

Peripheral blood was collected using BD CPT Vacutainers Cell Preparation Tubes with Sodium Heparin (BD, Franklin Lakes, NJ). The purification of buffy coat was carried out within 2 hr of blood draw. Each tube was centrifuged for 20 min at 1,720g at room temperature, the upper plasma layer was discarded and the lymphocyte and monocyte band transferred into a 15 ml falcon tube using a sterile transfer pipette. Ten milliliter of phosphate buffered saline (PBS) were added and the tubes were centrifuged for 15 min at 300g. The supernatant was discarded and the cell sediment again re-suspended in 15 ml PBS and centrifuged (10 min, 300g). After discarding the supernatant, the remaining cell pellet was re-suspended in 1.8 ml cell preservation medium (10% DMSO, 10 fetal calf serum, 80 DMEM) and stored at  $-80^{\circ}\text{C}$  until high molecular weight DNA isolation. High molecular weight genomic DNA was extracted using a QIAamp DNA blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions using ddH<sub>2</sub>O to elute DNA from the column. DNA concentration and quality were evaluated measuring the absorption ratio at 260/280 and 260/230 nm using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE) and standard agarose gel electrophoresis. The samples were diluted to 10 ng/ $\mu$ l for genotyping using the Sequenom MassARRAY system. Tag SNPs were selected using the HapMap database to characterize variation within each gene ( $\pm 5$  kilo-bases),

identifying variants with a frequency of at least 5%. Among SNPs in linkage disequilibrium ( $R^2 > 0.8$ ), we selected SNPs for analysis based on relevant function or previous literature.

### Outcome Assessment

The primary outcomes of interest were high-grade prostate cancer and prostate cancer recurrence. High-grade prostate cancer was defined as pathologic Gleason sum  $\geq 8$  or  $\geq 7$  with major Gleason score  $\geq 4$ . Sensitivity analyses were also conducted using only Gleason sum  $\geq 8$  as the outcome. Prostate cancer recurrence was defined as two or more consecutive PSA values  $> 0.2$  ng/ml more than 8 weeks after radical prostatectomy, initiation of secondary treatment  $\geq 6$  or more months after surgery, or metastases to bone.

### Clinical and Covariate Data

Data on age, race, treatment, biopsy Gleason sum, stage, and prostate specific antigen (PSA) were abstracted from medical records. Prognostic risk score was calculated according to modified D'Amico categories (high: PSA  $> 20$  ng/ml or Gleason sum  $> 7$  or T-stage  $\geq T3a$ ; else intermediate: PSA  $10.1$ – $20$  ng/ml or Gleason sum  $= 7$  or T-stage  $= T2b$ – $c$ ; low: PSA  $< 10$  ng/ml and Gleason sum  $< 7$  and T-stage  $\leq T2a$ ) [16]. We also calculated the CAPRA risk score, a clinically relevant and validated composite risk score, based on Gleason grade, PSA at diagnosis, clinical T-stage, and other pretreatment clinical data [17,18].

### Statistical Analysis

We collapsed the heterozygote and homozygous less common allele categories of SNPs when fewer than 5% of the study population was homozygous for the less common allele.

To examine the circulating  $\alpha$ - and  $\gamma$ -tocopherol and SNPs in relation to risk of high-grade prostate cancer, we used a logistic regression model adjusted for age at diagnosis (continuous) and race/ethnicity (Caucasian vs. non-Caucasian). Models examining circulating tocopherols were also adjusted for blood cholesterol (continuous) to remove extraneous variation in circulating tocopherol levels due to cholesterol levels measured in corresponding blood samples [19]. Plasma  $\alpha$ - and  $\gamma$ -tocopherol levels were categorized into quartiles and modeled using indicator variables with the lowest quartile as the reference category. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to assess the magnitude and direction of the associations. To test for evidence of a linear trend across tocopherol levels, we modeled the median of each quartile as a continuous ordinal variable and used a Wald test to determine the  $P$ -trend. We modeled the SNPs using additive models (an ordinal variable was used indicating the number of less common alleles = 0, 1, 2) and co-dominant models (indicator variables were used with the homozygous common allele as the reference) when sample size permitted, and used Wald tests to calculate the  $P$ -value for the additive models. In addition, we created a cross-product term between the circulating tocopherol levels (dichotomized at the median) and the SNPs (additive model) and used a Wald test to test for evidence of an interaction.

To examine the tocopherol levels and SNPs in relation to risk of prostate cancer recurrence, we used a Cox proportional hazards regression adjusting for age at diagnosis (continuous), race/ethnicity (Caucasian vs. non-Caucasian), and prognostic risk (low, intermediate, high).

Models examining circulating vitamin E levels were also adjusted for blood cholesterol (continuous) for the reason stated above. Person-time was calculated from date of surgery to date of recurrence, death from another cause, or June 2012, whichever came first. As above, we modeled quartiles of tocopherol levels using indicator variables and tested for evidence of a linear trend by modeling the median of each quartile as a continuous term. We also examined the SNPs in relation to risk of recurrence using additive and co-dominant models.

For all analyses, we performed a sensitivity test restricting to Caucasians. Analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary, NC) and two sided  $P$ -values  $< 0.05$  were considered statistically significant. Adjustment for multiple comparisons was not performed; thus, our results should be interpreted cautiously.

## RESULTS

Demographic and clinical characteristics of the study population, overall and by extreme quartiles, are listed in Table I. Patients with the highest levels of circulating  $\gamma$ -tocopherol were younger at diagnosis compared to patients with the lowest levels (57 years vs. 61 years,  $P < 0.0001$ ) and men with the highest levels of circulating  $\alpha$ -tocopherol were more likely to be Caucasian ( $P = 0.02$ ) than men with lower levels.

Higher levels of  $\gamma$ -tocopherol were suggestively associated with an increased risk of high-grade prostate cancer (Q4 v. Q1 OR = 1.87; 95% CI: 0.97, 3.58;  $P_{\text{trend}} = 0.02$ ; Table II). Using a stricter definition of high-grade disease (Gleason sum  $\geq 8$ ; events = 41), the magnitude of this association increased (Q4 v. Q1 OR = 2.06; 95% CI: 0.69, 6.15), but the test for linear trend was no longer statistically significant ( $P_{\text{trend}} = 0.10$ ). Plasma  $\alpha$ -tocopherol was not associated with risk of high-grade prostate cancer (Table II), and neither circulating  $\alpha$ - nor  $\gamma$ -tocopherol were associated with risk of prostate cancer recurrence (data not shown).

We observed no association between any SNP and risk of high-grade prostate cancer (Table III), with the exception of *SOD3* rs699473, which was associated with an increased risk of high-grade prostate cancer in the additive model (T > C: OR = 1.40, 95% CI: 1.04, 1.89). However, the increased risk was limited to the heterozygous genotype (TC v. TT (ref.): OR = 1.86, 95% CI: 1.17, 2.94) and this association was not robust in sensitivity analyses examining risk of Gleason sum  $\geq 8$  (data not shown).

SNPs rs17884057 and rs9967983 in *SOD1* were inversely associated with time to prostate cancer recurrence (HR per risk allele (AGA deletion) = 0.49, 95% CI: 0.25, 0.96 and HR per risk allele (T) = 0.62, 95% CI: 0.40, 0.95, respectively; Table III). In the co-dominant model, the TT genotype of rs9967983 in *SOD1* was associated with a 78% decreased risk of prostate cancer recurrence compared to the reference AA genotype (HR = 0.22, 95% CI: 0.06, 0.76; Table IV). In addition, two SNPs from *SOD2* were moderately associated with a decreased risk of prostate cancer recurrence in the co-dominant model. The heterozygous TC genotype of SNP rs4880 was associated with a 53% decreased risk of prostate cancer recurrence compared to the reference common allele (HR = 0.47, 95% CI: 0.25, 0.88). The effect estimate for the homozygous rare TT allele (n = 31) was in the same direction, but did



not reach statistical significance (HR = 0.57, 95% CI: 0.28, 1.16). Similarly, SNP rs2758330 in *SOD2* was inversely associated with prostate cancer recurrence, although only the heterogeneous genotype reached statistical significance (TG v. TT: HR = 0.40, 95% CI: 0.19, 0.82; GG v. TT: HR = 1.09; 95% CI: 0.38, 3.13). SNPs in *TTPA* and *SEC14L2* were not associated with risk of prostate cancer recurrence.

We observed a statistically significant interaction between the *SOD1* rs17884057 variant, circulating  $\alpha$ -tocopherol levels, and risk of high-grade prostate cancer, although our data were sparse ( $P_{\text{interaction}} = 0.03$ ). Among 392 men with the AGA/AGA genotype, the highest quartile of circulating  $\alpha$ -tocopherol was associated with a non-statistically significant increased risk of high-grade prostate cancer (OR = 1.46, 95% CI: 0.66, 3.22). Conversely, among 156 men with a single or double deletion, the highest quartile of circulating  $\alpha$ -tocopherol trended towards a decreased risk of prostate cancer (OR = 0.54, 95% CI: 0.15, 2.00).

Results were similar when restricting to Caucasian men.

## DISCUSSION

In this case-only study among men initially diagnosed with clinically organ-confined prostate cancer and treated via radical prostatectomy, both higher levels of circulating  $\gamma$ -tocopherol and the less common allele in rs699473, a SNP in *SOD3*, were associated with an increased risk of high-grade prostate cancer. In addition, SNPs in *SOD1* (rs17884057 and rs9967983) and *SOD2* (rs2758330 and rs4880) were associated with decreased risks of prostate cancer recurrence after radical prostatectomy. These results remained statistically significant when adjusting for age at diagnosis, prognostic risk at diagnosis, and race/ethnicity. Overall, our results should be interpreted cautiously given our limited sample size and the possibility of chance findings due to multiple testing, thus confirmation is needed in multiple independent populations.

Evidence for an association between  $\gamma$ -tocopherol and prostate cancer risk is conflicting. Consistent with our findings, a recent nested case-control study in the PLCO Screening Trial observed a trend towards increased risk of prostate cancer among men in the highest quintile of circulating  $\gamma$ -tocopherol (OR = 1.35, 95% CI: 0.92, 1.97), however this association appeared to be limited to less aggressive tumors [20]. Of the 11 other cohort studies examining the association between  $\gamma$ -tocopherol and prostate cancer risk, three observed a reduced risk of prostate cancer incidence among men with higher levels of circulating  $\gamma$ -tocopherol [21–23] and eight observed no association [24–31]. This is the first study to examine the relationship between  $\alpha$ - and  $\gamma$ -tocopherol levels and risk of high-grade prostate cancer in a case-only study.

Two independent *SOD1* SNPs (rs17884057 and rs9967983) were inversely associated with prostate cancer recurrence. Both SNPs are intronic, and thus it is possible that these are in linkage disequilibrium with functional *SOD1* SNPs, however, they were not correlated with each other ( $r = 0.44$ ). For *SOD2*, the less common allele was protective in both rs2758330 and rs4880, but the homozygous genotype was not statistically significant for either SNP,

possibly due to the small sample size. *SOD2* rs2758330 is an intronic mutation and rs4880 is a missense mutation, likely leading to a dysfunctional *SOD2* protein. None of these SNPs were in linkage disequilibrium ( $R^2 < 0.8$ ).

Previous studies of *SOD* have focused on risk of incident prostate cancer and aggressive disease, but have not examined risk of prostate cancer recurrence. In one of the only other case-only analyses, men with at least one deletion in *SOD1* rs17884057 had a 17% decreased risk of aggressive prostate cancer at diagnosis (RR = 0.83, 95% CI: 0.77, 0.99) [32]. The two *SOD2* SNPs associated with prostate cancer recurrence in this study (rs2758330 and rs4880) have been found to modify the relationship between circulating selenium levels, also an antioxidant, and high-grade prostate cancer in previous studies [12,32,33]. A recent meta-analysis examining the relationship between SNPs of antioxidant genes and various diseases reported a 16% increased risk of prostate cancer among men with the Ala/Ala (CC) and Val/Ala (TC) genotypes of *SOD2* rs4880 from a pooled analysis of 10 studies (OR = 1.16, 95% CI: 1.03, 1.32) [34]. Furthermore, an increased risk of high-grade tumors was observed in men with the Ala allele [35], especially in the presence of high selenium or low lycopene levels [12,36]. Previous studies have not observed an association between *SOD3* and the risk of prostate cancer or aggressive tumors [11,32].

Superoxide dismutase (*SOD*) enzymes protect cells from oxidative stress and toxicity. Decreased expression of *SOD* genes in prostatic intraepithelial neoplasia may lead to increased susceptibility to oxidative damage and malignancy [10]. Experimental studies have shown that oxidative damage from reactive oxygen species may play an important role in the development and recurrence of prostate cancer through pathways involving inflammation, antioxidant defense systems, and regulation of androgens [37]. Specifically, in vitro studies suggest that oxidative stress may be required for the development of aggressive phenotypes in prostate cancer cells [38]. Several recently discovered genes may modulate the relationship between oxidative stress and prostate cancer recurrence, however these preliminary results must be confirmed in large, epidemiologic studies [39–43]. *SOD2* has also shown some potential as a tumor suppressor gene, through apoptotic and anti-proliferative mechanisms [9].

This study has several limitations. First, we observed a limited number of recurrence outcomes and had a limited sample size to test for evidence of interactions. Second, this analysis was conducted among primarily Caucasian men, thus our results may not be generalizable to populations with different race/ethnicity distributions. Third, we were limited to the available data on possible confounders, which did not include smoking, dietary, or physical activity data. However, the relations observed were for germline genetic variants, which are unlikely to be confounded by lifestyle factors. We acknowledge the possibility that observed associations may be due to linkage disequilibrium with genetic variants that were not measured as well as the potential for false positives due to multiple testing. Therefore, these results must be viewed as exploratory and provide rationale for further investigations of the role of antioxidants and related genetic variants in prostate cancer recurrence.



In conclusion, germline genetic variation in *SOD* genes may be associated with the risk of prostate cancer recurrence after radical prostatectomy among men with clinically organ-confined prostate cancer. Further research is needed to assess the value of incorporating genetic information into prognostic risk scores. Consideration of *SOD* genotype may prove useful in the interpretation of vitamin E supplementation trials such as SELECT.

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Descriptive Statistics of 573 Men Initially Diagnosed With Organ-Confining Prostate Cancer, Overall and by Extreme Quartiles of Circulating Alpha- and Gamma-Tocopherol\*

TABLE I

Variable	Total N (%)	Extreme quartiles of plasma alpha-tocopherol			Extreme quartiles of plasma gamma-tocopherol		
		Q1	Q4	P-value <sup>d</sup>	Q1	Q4	P-value <sup>d</sup>
Number of participants	573	139	140		140	139	
Age at diagnosis, years, mean ± SD		58.6 ± 7.3	60.2 ± 6.1	0.06	60.7 ± 7.0	57.0 ± 6.9	<0.0001
PSA at diagnosis, ng/ml, mean ± SD		7.8 ± 10.0	7.7 ± 6.3	0.57	7.5 ± 5.8	8.4 ± 10.4	0.13
Serum tocopherol <sup>b</sup> , µg/ml, mean ± SD		8.5 ± 1.8	19.4 ± 4.3		0.46 ± 0.1	2.3 ± 1.0	
Caucasian, %	497 (87)	79	88	0.02	86	88	0.85
Clinical stage, %				0.92			0.22
T2a/T2b	144 (25)	29	28		20	21	
T2c	300 (52)	49	49		62	53	
T3a/T3b	129 (23)	22	24		18	27	
Gleason score, %				0.08			0.28
<7	204 (36)	38	31		34	33	
7	328 (57)	53	63		61	58	
>7	41 (7)	9	6		5	9	
Prognostic Risk Group <sup>c</sup> , %				0.63			0.28
Low	60 (10)	12	14		9	7	
Intermediate	328 (57)	55	51		63	55	
High	185 (32)	33	35		28	37	
CAPRA score, %				0.06			0.37
<3 (low)	180 (31)	32	28		34	30	
3–5 (intermediate)	146 (25)	24	27		24	28	
>5 (high)	29 (5)	9	4		4	6	

\* Quartiles calculated using the log of circulating alpha- and gamma-tocopherol.

<sup>d</sup> P-value calculated from a logistic model examining quartiles of alpha- and gamma-tocopherol as the dependent variables and the characteristic of interest as the independent variable. The Type III P-value was used for categorical independent variables and the Wald test P-value was used for continuous independent variables.

<sup>b</sup> Fourteen study participants with missing data on plasma tocopherols were excluded from all analyses.

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Low = Gleason sum < 7, PSA at diagnosis < 10 ng/ml, AND clinical T-stage = T2a or less; Intermediate = Gleason sum < 7, PSA at diagnosis = 10–19.9 ng/ml, AND clinical T-stage < T3a OR Gleason sum = 7, PSA at diagnosis < 20 ng/ml, AND clinical T-stage < T3a; High = Gleason sum > 7, PSA at diagnosis ≥ 20 ng/ml, OR clinical T-stage = T3a or higher.

TABLE II

Relative Risk of High-Grade Prostate Cancer Among 573 Men Initially Diagnosed With Organ-Confined Disease, by Circulating Alpha- and Gamma-Tocopherol

	No. of events <sup>a</sup>	Multivariate OR (95% CI) <sup>b</sup>	P-trend <sup>c</sup>
Quartile of plasma alpha-tocopherol			
1	32	1.0 (ref.)	
2	21	0.66 (0.35–1.26)	
3	29	1.02 (0.54–1.93)	
4	31	1.12 (0.57–2.17)	0.61
Quartile of plasma gamma-tocopherol			
1	24	1.0 (ref.)	
2	24	1.15 (0.61–2.19)	
3	33	1.84 (0.99–3.43)	
4	32	1.87 (0.97–3.58)	0.02

<sup>a</sup>Total Gleason = 8 or Gleason = 7 with primary score = 4.

<sup>b</sup>Multivariate logistic regression model adjusted for age at diagnosis (years), blood cholesterol (mg/dl), race/ethnicity (Caucasian vs. non-Caucasian).

<sup>c</sup>P-trend calculated by modeling the median of each quartile as a continuous term.



**TABLE III**  
Relative Risk of High-Grade Prostate Cancer and Prostate Cancer Recurrence Among 573 Men Initially Diagnosed With Organ-Confining Disease (Additive Model)

	Allele	MAF <sup>a</sup>	High-grade prostate cancer (events = 117)			Prostate cancer recurrence (events = 56)		
			OR <sup>b</sup>	95% CI	P-trend	HR <sup>c</sup>	95% CI	P-value
<b>SOD1</b>								
rs10432782	T > G	0.15	1.27	0.80–2.00	0.32	0.76	0.39–1.52	0.44
rs17884057	AGA > del <sup>d</sup>	0.16	1.11	0.71–1.74	0.65	0.49	0.25–0.96	0.04
rs4816407	A > G <sup>d</sup>	0.10	1.24	0.72–2.12	0.44	0.74	0.32–1.71	0.48
rs9967983	A > T	0.46	1.22	0.89–1.67	0.21	0.62	0.40–0.95	0.03
rs2070424	A > G <sup>d</sup>	0.11	1.02	0.60–1.73	0.96	0.57	0.24–1.38	0.21
<b>SOD2</b>								
rs2758330	T > G	0.22	1.16	0.83–1.61	0.40	0.66	0.39–1.10	0.11
rs4880	C > T	0.49	1.15	0.86–1.53	0.35	0.71	0.48–1.04	0.07
rs5746136	G > A	0.28	1.00	0.73–1.39	0.98	0.86	0.55–1.32	0.48
rs5746138	A > G <sup>d</sup>	0.04	1.16	0.57–2.35	0.68	0.90	0.35–2.29	0.83
rs6917589	T > C	0.23	1.01	0.72–1.41	0.96	0.87	0.55–1.37	0.54
rs7855	A > G	0.05	0.73	0.33–1.60	0.43	0.20	0.03–1.43	0.11
<b>SOD3</b>								
rs1007991	G > C	0.34	1.20	0.89–1.62	0.22	1.11	0.75–1.63	0.62
rs17881426	T > A <sup>d</sup>	0.09	0.92	0.53–1.62	0.78	0.75	0.32–1.77	0.52
rs699473	T > C	0.36	1.40	1.04–1.89	0.03	1.07	0.72–1.58	0.75
rs8192287	G > T <sup>d</sup>	0.05	0.79	0.37–1.67	0.54	0.70	0.22–2.29	0.56
rs8192291	C > T	0.19	1.12	0.72–1.73	0.61	1.36	0.78–2.38	0.28
<b>TTPA</b>								
rs6994076	A > T	0.48	1.04	0.78–1.40	0.77	1.10	0.75–1.63	0.63
rs7818611	A > G <sup>d</sup>	0.09	0.75	0.43–1.32	0.32	0.66	0.29–1.48	0.31
rs7818905	G > A	0.26	1.07	0.76–1.50	0.72	0.87	0.55–1.39	0.56
rs7842218	G > T <sup>d</sup>	0.03	0.95	0.40–2.23	0.91	0.97	0.30–3.12	0.96
<b>SEC14L2</b>								

	High-grade prostate cancer (events = 117)				Prostate cancer recurrence (events = 56)			
	Allele	MAF <sup>a</sup>	OR <sup>b</sup>	95% CI	P-trend	HR <sup>c</sup>	95% CI	P-value
rs1010324	G > A <sup>d</sup>	0.19	0.94	0.60–1.45	0.76	0.58	0.31–1.08	0.09
rs1061664	G > A <sup>d</sup>	0.19	1.13	0.74–1.74	0.58	1.11	0.64–1.94	0.71
rs2299825	A > G <sup>d</sup>	0.21	1.00	0.64–1.56	0.99	1.39	0.77–2.51	0.27
rs2299826	C > T <sup>d</sup>	0.10	0.82	0.48–1.41	0.48	1.09	0.57–2.08	0.79
rs2299829	T > A	0.50	1.10	0.83–1.47	0.49	1.10	0.75–1.61	0.62
rs3216411	G > del	0.28	1.03	0.74–1.42	0.87	1.01	0.66–1.55	0.97
rs757660	G > A	0.30	0.94	0.70–1.28	0.71	1.20	0.81–1.77	0.36
rs887098	G > A	0.27	0.97	0.70–1.33	0.83	1.29	0.88–1.91	0.20

<sup>a</sup>Minor allele frequency (MAF) calculated using allelic distribution of the study population.

<sup>b</sup>Multivariate logistic regression model adjusted for age at diagnosis (years) and race/ethnicity (Caucasian vs. non-Caucasian).

<sup>c</sup>Survival model adjusted for age at diagnosis (years), prognostic risk score (low = Gleason sum < 7, PSA at diagnosis < 10 ng/ml, AND clinical T-stage = T2a or less; intermediate = Gleason sum < 7, PSA at diagnosis = 10–19.9 ng/ml, AND clinical T-stage < T3a OR Gleason sum = 7, PSA at diagnosis < 20 ng/ml, AND clinical T-stage < T3a; High = Gleason sum > 7, PSA at diagnosis ≥ 20 ng/ml, OR clinical T-stage = T3a or higher), race/ethnicity (Caucasian vs. non-Caucasian).

<sup>d</sup>Homozygous rare genotype less than 5% of cohort, thus heterozygote combined with homozygous rare genotype.

**TABLE IV**

Relative Risk of High-Grade Prostate Cancer and Prostate Cancer Recurrence Among a Clinical Cohort of 573 Men Initially Diagnosed With Organ-Confined Disease (Co-Dominant Model)

Gene/SNP	Allele	N	High grade prostate cancer (events = 117)			Prostate cancer recurrence (events = 56)		
			OR <sup>a</sup>	95% CI	P-value	HR <sup>b</sup>	95% CI	P-value
<b>SOD1</b>								
rs9967983	AA	148	1.0 (ref.)			1.0 (ref.)		
	AT	260	1.30	0.78–2.19	0.31	0.95	0.51–1.75	0.86
	TT	103	1.48	0.79–2.78	0.22	0.22	0.06–0.76	0.02
<b>SOD2</b>								
rs2758330	TT	347	1.0 (ref.)			1.0 (ref.)		
	TG	187	1.13	0.73–1.75	0.59	0.40	0.19–0.82	0.01
	GG	31	1.40	0.59–3.28	0.44	1.09	0.38–3.13	0.88
rs4880	CC	150	1.0 (ref.)			1.0 (ref.)		
	CT	276	0.91	0.55–1.51	0.73	0.47	0.25–0.88	0.02
	TT	134	1.31	0.75–2.29	0.35	0.57	0.28–1.16	0.12
rs5746136	GG	297	1.0 (ref.)			1.0 (ref.)		
	GA	224	0.96	0.62–1.47	0.84	0.90	0.52–1.58	0.72
	AA	45	1.08	0.51–2.32	0.83	0.65	0.20–2.14	0.48
rs6917589	TT	336	1.0 (ref.)			1.0 (ref.)		
	TC	198	0.91	0.59–1.42	0.68	0.83	0.46–1.49	0.63
	CC	32	1.24	0.53–2.88	0.63	0.83	0.25–2.71	0.75
<b>SOD3</b>								
rs1007991	GG	255	1.0 (ref.)			1.0 (ref.)		
	GC	229	1.22	0.78–1.92	0.38	1.18	0.66–2.12	0.57
	CC	73	1.43	0.76–2.70	0.27	1.16	0.49–2.74	0.74
rs699473	TT	236	1.0 (ref.)			1.0 (ref.)		
	TC	249	1.86	1.17–2.94	0.009	1.31	0.73–2.36	0.36
	CC	76	1.72	0.90–3.29	0.10	0.97	0.38–2.43	0.94
<b>TTPA</b>								
rs6994076	AA	151	1.0 (ref.)			1.0 (ref.)		

Gene/SNP	Allele	N	High grade prostate cancer (events = 117)			Prostate cancer recurrence (events = 56)		
			OR <sup>a</sup>	95% CI	P-value	HR <sup>b</sup>	95% CI	P-value
rs7818905	AT	285	1.22	0.74–2.01	0.44	1.09	0.57–2.11	0.79
	TT	128	1.08	0.59–1.97	0.81	1.21	0.56–2.64	0.63
	GG	280	1.0 (ref.)			1.0 (ref.)		
SEC14L2	GA	197	1.02	0.65–1.60	0.94	0.62	0.33–1.18	0.15
	AA	37	1.22	0.54–2.75	0.63	1.22	0.47–3.17	0.69
	TT	151	1.0 (ref.)			1.0 (ref.)		
rs2299829	TA	257	0.82	0.50–1.36	0.44	0.55	0.28–1.05	0.07
	AA	150	1.21	0.70–2.09	0.50	1.25	0.64–2.42	0.51
	GG	287	1.0 (ref.)			1.0 (ref.)		
rs3216411	G/-	234	0.81	0.52–1.26	0.35	0.86	0.48–1.53	0.61
	-/-	42	1.47	0.71–3.04	0.31	1.28	0.49–3.31	0.62
	GG	277	1.0 (ref.)			1.0 (ref.)		
rs757660	GA	210	0.76	0.49–1.19	0.24	1.00	0.55–1.83	0.86
	AA	61	1.08	0.56–2.07	0.82	1.59	0.73–3.44	0.24
	GG	306	1.0 (ref.)			1.0 (ref.)		
rs887098	GA	199	0.87	0.56–1.36	0.55	1.28	0.72–2.29	0.40
	AA	48	1.06	0.51–2.21	0.87	1.68	0.72–3.91	0.23
	GG	306	1.0 (ref.)			1.0 (ref.)		

<sup>a</sup> Multivariate logistic regression model adjusted for age at diagnosis (years) and race/ethnicity (Caucasian vs. non-Caucasian).

<sup>b</sup> Survival model adjusted for age at diagnosis (years), prognostic risk score (low = Gleason sum < 7, PSA at diagnosis < 10 ng/ml, AND clinical T-stage = T2a or less; intermediate = Gleason sum < 7, PSA at diagnosis = 10–19.9 ng/ml, AND clinical T-stage < T3a OR Gleason sum = 7, PSA at diagnosis < 20 ng/ml, AND clinical T-stage < T3a; high = Gleason sum > 7, PSA at diagnosis ≥ 20 ng/ml, OR clinical T-stage = T3a or higher), race/ethnicity (Caucasian vs. non-Caucasian).