Research Article

Cancer Epidemiology, Biomarkers & Prevention

Oxidative Stress–Related Genetic Variants, Pro- and Antioxidant Intake and Status, and Advanced Prostate Cancer Risk

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Abstract

Background: Increased oxidative stress has been linked to prostate cancer. We investigated oxidative stress–related genetic variants in relation to advanced prostate cancer risk and examined potential interactions with pro- and antioxidant exposures.

Methods: A case-cohort analysis was conducted in the prospective Netherlands Cohort Study, which included 58,279 men ages 55 to 69 years. Cohort members completed a baseline questionnaire and provided toenail clippings, which were used to isolate DNA. Advanced prostate cancer cases were identified during 17.3 years of follow-up. The analysis included 14 genetic variants and 11 exposures. Cox regression models were used for analysis and FDR *Q*-values were calculated.

Results: Complete genotyping data were available for 952 cases and 1,798 subcohort members. *CAT* rs1001179 was associated with stage III/IV and stage IV prostate cancer risk, with HRs per minor allele of 1.16 [95% confidence intervals (CI), 1.01–1.33; $P = 0.032$] and 1.25 (95% CI, 1.07–1.46; $P = 0.006$), respectively. We tested 151 gene–environment interactions in relation to both stage III/IV and IV prostate cancer risk. Seven interactions were statistically significant after adjusting for multiple testing (FDR *Q*-value <0.20); for stage III/IV prostate cancer, these involved intake of β-carotene (*GPX1* rs17650792, *hOGG1* rs1052133) and heme iron (*GPX1* rs1800668 and rs3448), and for stage IV prostate cancer, these involved intake of catechin (*SOD2* rs4880) and heme iron (*hOGG1* rs1052133, *SOD1* rs10432782).

Conclusion: This study of advanced prostate cancer risk showed a marginal association with a *CAT* polymorphism and seven novel gene–environment interactions in the oxidative stress pathway.

Impact: Oxidative stress–related genes and exposures may have a joint effect on advanced prostate cancer. *Cancer Epidemiol Biomarkers Prev; 24(1); 178–86. ©2014 AACR.*

Introduction

Oxidative stress results from an imbalance between antioxidant protection and reactive oxygen species (ROS) produced by pro-oxidants (1, 2). ROS are strong oxidizing agents that cause damage to cellular constituents when levels are elevated (1, 2). Antioxidants include endogenous enzymes (e.g., superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase; GPX) and exogenous dietary nutrients (e.g., vitamins C and E, carotenoids, flavonoids, and selenium; refs. 3, 4). Important pro-oxidant factors include inflammatory processes and the mitochondrial electron transport chain, but also exogenous exposures such as cigarette smoking, dietary iron, and radiation (2, 3). Oxidative stress becomes more common with advancing age (5), and has been implicated in age-related degenerative diseases, including prostate cancer (1, 6, 7).

Previous epidemiologic studies on antioxidant nutrients and prostate cancer risk have generated mixed results (8). More than 50 observational (prospective and case–control) studies investigated the associations of the major dietary antioxidants vitamin C and E, lycopene, β-carotene, and selenium. Results from these studies showed that most antioxidants were not associated (8, 9), with the exception of selenium, which has been inversely associated with both overall and advanced prostate cancer risk in many studies (10), including our study (11, 12). Secondary results from two randomized controlled trials, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study (13), and the Nutritional Prevention of Cancer (NPC) study (14), showed prostate cancer risk reductions for vitamin E and selenium, respectively. These findings led to the design of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which, however, showed no protective effect of both nutrients (15). There have been a number of observational studies that investigated pro-oxidant exposures such as smoking, alcohol, and dietary iron in relation to overall and advanced prostate cancer risk, but these studies do not support an association (16–20).

Genetic variation in genes related to oxidative stress may influence prostate cancer risk. These genes include those encoding pro- and antioxidant enzymes but also other oxidative stress–related genes (e.g., oxidative DNA damage repair). Several variants in these genes have been identified that have potential functional consequences (21–26). Candidate gene-association studies investigated these genetic variants in relation to prostate cancer risk and showed significant associations for variants in different genes such as *SOD2*, *PON1*, *GPX1*, *NOS2A*, *NOS3*, and *hOGG1* (21–26). Genome-wide association studies (GWAS) of prostate cancer, however, have not identified risk variants in major oxidative

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doi: 10.1158/1055-9965.EPI-14-0968

stress–related genes (27). Besides having a main effect on prostate cancer risk, oxidative stress–related gene variants may also interact with pro- and antioxidant exposures and potentially modify their effects. This has been investigated in a small number of studies that focused on specific a priori defined gene–environment interactions. These studies have produced statistically significant results, including the interactions between *SOD2* and selenium (28), *SOD2* and iron (16), and *hOGG1* and α-tocopherol (25).

In the present study, we evaluated 14 genetic variants in oxidative stress–related genes. The gene variants are SNPs reported in prior studies to have either a functional effect or an association with prostate cancer risk. We investigated associations between these SNPs and advanced prostate cancer risk and examined potential gene–environment interactions with major pro- and antioxidant exposures. We specifically studied advanced prostate cancer, which is a subtype of prostate cancer associated with a poor prognosis that is, therefore, clinically relevant (29).

Materials and Methods

Study population and design

The prospective Netherlands Cohort Study included 58,279 men, ages 55 to 69 years at baseline in September 1986. Study participants completed a baseline questionnaire on dietary habits, lifestyle, health, and several other potential cancer risk factors (30). Approximately 81% of cohort members provided toenail clippings from all ten toes, which were used to determine toenail selenium levels and to isolate DNA for genotyping. Cancer cases were identified by annual record linkage to the Netherlands Cancer Registry and the Dutch Pathology Registry (31). The completeness of follow-up through linkage with these registries was assessed to be at least 96% (32). The Netherlands Cohort Study uses the case-cohort design (33); cases are derived from the entire cohort and the person-time experience is estimated from a subcohort randomly sampled from the full cohort at baseline ($n = 2{,}411$). All participants with prevalent cancer other than skin cancer at baseline were excluded (approximately 3%). Figure 1 shows a flow diagram of cases and subcohort members. The Netherlands Cohort Study has been approved by the Institutional Review Boards of the TNO Nutrition and Food Research Institute (Zeist, the Netherlands) and Maastricht University (Maastricht, the Netherlands).

Ascertainment and classification of participants who developed prostate cancer

During 17.3 years of follow-up (September 1986 to December 2003), 3,667 incident prostate cancer cases (ICD-O-3 code C61) were identified. Prostate cancer cases were classified on the basis of tumor-node-metastasis (TNM) staging (pathologic TNM, where available, or clinical TNM; ref. 34). In the present analysis, we specifically evaluated advanced prostate cancer (International Union Against Cancer, stage III and IV; ref. 34), which included tumors with T3–4, N+, or M1 at diagnosis ($n = 1{,}290$; Fig. 1). Stage IV prostate cancer is a subset of advanced prostate cancer and was stage T4, N+, or M1 at diagnosis ($n = 817$). A number of cases had missing data on tumor stage ($n = 235$) and were excluded from the analysis.

DNA isolation, gene variant selection, and genotyping

DNA was isolated from available toenail samples using a phenol-chloroform extraction (23, 35). We previously showed that toenail samples are a long-term stable source of DNA for genotyping (35). The minimum amount of toenail material required for DNA extraction was 4 mg and we excluded participants who provided less. Toenail DNA was successfully isolated for 1,030 cases and 1,885 subcohort members (>99.9% successful DNA isolation; Fig. 1).

We selected SNPs in oxidative stress–related genes reported in prior studies to have a functional effect (e.g., altered gene expression or enzyme activity) or an association with prostate cancer risk. The following SNPs were selected: *CAT* rs1001179 (36), *GPX1* rs17650792 (23), *GPX1* rs1800668 (23), *GPX1* rs3448 (37), *hOGG1* rs1052133 (25), *NOS2A* rs2297518 (24), *NOS2A* rs9282801 (24), *NOS3A* rs1799983 (24), *NQ1* rs1800566 (38), *PON1* rs662 (22), *PON1* rs854560 (22), *SOD1* rs10432782 (39), *SOD2* rs4816407 (39), and *SOD2* rs4880 (21). Selection of genetic variants was restricted to those with a minor allele frequency of at least 10%. *MPO* rs2333227 was not included in the final SNP selection because it was not possible to design unique primers for the SNP given the other SNPs selected in our study.

SNP genotyping was done by designing PCR assays and extension primers with the use of MassARRAY software, version 4.0 (Sequenom Inc.). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry with the use of the iPLEX Gold system (Sequenom Inc.).

Quality control included genotyping of blind duplicate samples ($n = 143$, or 5% of all genotyped samples), which revealed >99% agreement on genotyping calls across all SNPs assayed. Samples with a sample call rate (i.e., percentage of called SNPs per sample) of less than 95% were excluded (78 cases and 87 subcohort members) and 952 cases and 1,798 subcohort members had complete genotyping data (Fig. 1). All SNPs had >99% genotype completion rates (i.e., percentage of non-missing samples per SNP). None of the SNPs violated Hardy–Weinberg equilibrium ($P > 0.05$).

Exposure assessment

A baseline food frequency questionnaire (FFQ) was used to assess dietary pro- and antioxidant intake (40, 41). The FFQ was part of a larger questionnaire that also included questions about various other factors such as lifestyle, family history of cancer, and health. We selected and studied the major dietary antioxidant nutrients: β-carotene, lycopene, catechin, vitamin C, and vitamin E (intake in mg/day); and the major pro-oxidant factors: heme iron (intake in mg/day), alcohol (intake in g/day), and smoking status (never, former, current; ref. 17). We also evaluated an antioxidant score (AOS) and oxidative balance score (OBS), which have been investigated in previous studies (17). In our study, the AOS is the sum of quartile scores (Q1 = 0; Q2 = 1; Q3 = 2; Q4 = 3) of intake of β-carotene, lycopene, catechin, vitamin C, and vitamin E. The OBS additionally includes heme iron intake (Q1 = 3; Q2 = 2; Q3 = 1; Q4 = 0), alcohol consumption (abstainer = 3; 0.1–4 g/day = 2.25; 5–14 g/day = 1.5; 15–29 g/day = 0.75; ≥30 g/day = 0), and smoking status (never = 3, former = 1.5, current = 0); these pro-oxidants are scored in the opposite way to the antioxidants.

The FFQ was validated and tested for reproducibility. Comparison with a 9-day dietary record showed that it was able to rank subjects adequately according to intake of the food groups and nutrients investigated (40). The FFQ was also found to be a good indicator of nutrient intake over a period of at least 5 years (41).

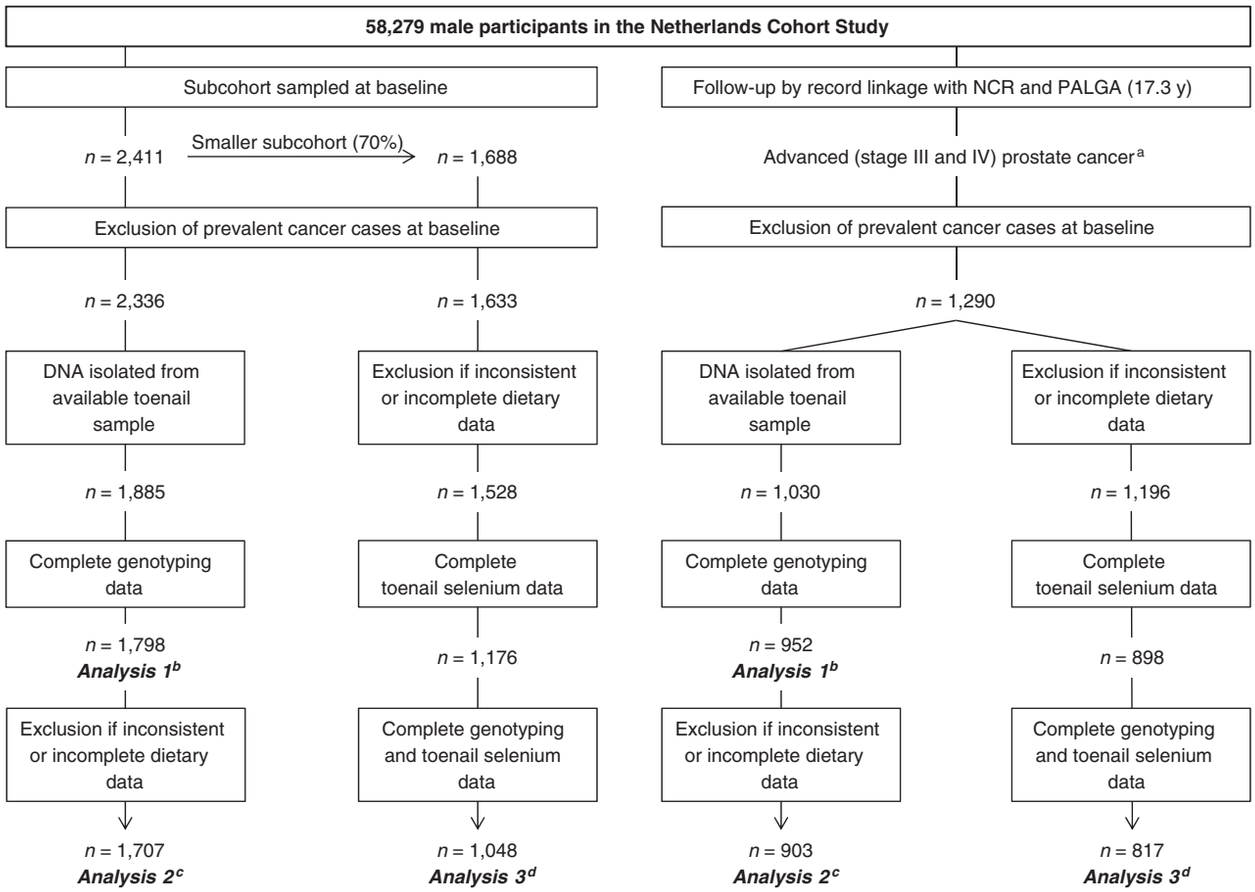

Figure 1.
Flow diagram of advanced prostate cancer cases and subcohort members, Netherlands Cohort Study, 1986–2003. NCR, Netherlands Cancer Registry; PALGA, Dutch Pathology Registry. [a]Advanced prostate cancer was defined as T3–4, N+, or M1 at diagnosis (International Union Against Cancer, stage III/IV). On average, 63% of advanced cases were stage IV prostate cancer (stage T4, N+, or M1 at diagnosis). [b]Analysis 1 is on the associations between oxidative stress–related genetic variants and advanced prostate cancer risk. The analysis included 952 cases and 1,798 subcohort members. [c]Analysis 2 is on potential interactions between oxidative stress–related genetic variants and pro- and antioxidant intake on advanced prostate cancer risk. The analysis included 903 cases and 1,707 subcohort members. [d]Analysis 3 is on potential interactions between oxidative stress–related genetic variants and toenail selenium levels on advanced prostate cancer risk. The analysis included 817 cases and 1,048 subcohort members. A smaller version of the subcohort (70%) was used for this analysis.

Concentrations of specific nutrients in foods and beverages were derived from food composition tables based on data gathered in the Netherlands (17, 42). We excluded participants with incomplete or inconsistent dietary data, according to criteria described previously; 903 advanced prostate cancer cases and 1,707 subcohort members had complete genotyping and dietary intake data (Fig. 1; ref. 40).

Toenail selenium measurements

Selenium concentrations were measured in toenail samples using instrumental neutron activation analysis of the ^{77m}Se isotope (11, 12). Each sample went through six cycles of 17-second irradiation at a thermal neutron flux of 3×10^{16} $m^{-2}s^{-1}$, 3-second decay, and 17-second counting at 1 cm from a 40% germanium detector. The accuracy of the analysis was checked by a certified bovine liver standard (Standard Reference Material 1577b of the US National Institute of Standards and Technology). We excluded participants who provided a toenail sample of less than 10 mg because the selenium determination could otherwise be unreliable. A smaller version of the subcohort was used for the toenail selenium determination (70%). The final analysis included 817 cases and 1,048 subcohort members who had complete toenail selenium data and genotyping data (Fig. 1).

Statistical analysis

The risk of advanced (stage III/IV and IV) prostate cancer in relation to oxidative stress–related genetic variants was assessed by age-adjusted Cox proportional hazards regression models. The SNPs were analyzed under a codominant and log-additive genetic model. The SEs were estimated by using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort (43). The proportional hazards assumption was tested using the scaled Schoenfeld residuals (44). In none of the analyses, the proportional hazards assumption was violated. The associations between the three *GPX1* SNPs (rs17650792, rs1800668, and rs3448) and advanced prostate cancer risk have been reported previously by our group (23), and are therefore not part of the present analysis.

We tested for multiplicative interactions between pro- and antioxidant exposures (quartiles or categories) and genotypes (dominant model) on advanced prostate cancer (stage III/IV and IV) risk by using cross-product terms in the regression models and the Wald statistic. Exposure quartiles were based on the distribution of the variable in the subcohort. We used a dominant genetic model to increase study power, unless there was evidence of a recessive association in our study or previous studies. Interactions between *GPX1* SNPs (rs17650792, rs1800668, and rs3448) and toenail selenium levels on advanced prostate cancer risk have been reported previously by our group (23), and are therefore not part of the present analysis. The following factors were assessed as potential confounding variables: family history of prostate cancer among first-degree relatives (yes/no), smoking status (never, former, or current), duration of smoking (years), frequency of smoking (cigarettes/day), nonoccupational physical activity (≤30, >30–60, >60–90, >90 minutes/day), body mass index (<23, 23–<25, 25–<27, 27–<30, ≥30), height (cm), diabetes (type I or II, yes/no), education level (primary school, lower vocational, high school, higher vocational/university), and intakes of energy (kcal/day) and calcium (g/day). These variables were identified as confounders when adding them, one at a time, to the age-adjusted model changed the point estimates by at least 10%, which was the case for none of these variables.

To account for multiple statistical testing when identifying gene–environment interactions (total number of tests performed = 302), we calculated FDR *Q*-values (45, 46). The FDR *Q*-value represents the expected proportion of false-positive results when testing for significance. FDR *Q*-values are calculated by multiplying the $P_{interaction}$ (calculated from each test from regression models) by the number of tests performed; and then dividing the multiplication by the rank order of each *P* value (where rank order 1 is assigned to the smallest *P* value; refs. 46, 47). We calculated an FDR *Q*-value for each pro- and antioxidant-specific interaction accounting for the total number of SNPs under investigation. We used an FDR *Q*-value threshold of 0.20, which has been suggested for candidate gene studies (46). A more stringent threshold is often used in GWAS, which typically do not use prior (mechanistic) information to select candidate genes (48). In this manuscript, we presented SNP–environment interactions with a *P* for multiplicative interaction <0.05. For each statistical interaction, we report the FDR *Q*-value and the HRs from the stratified analysis using a single reference category (lowest quartile or category of exposure and the common homozygote genotype).

All tests were two sided with a *P* value of <0.05 considered to be statistically significant. Analyses were performed using STATA software (release 12, STATA Corporation).

Results

Table 1 shows the baseline characteristics of advanced prostate cancer cases and subcohort members. Compared with subcohort members, cases were more likely to have a first-degree relative with prostate cancer and less likely to have a history of diabetes.

Table 2 shows the associations between oxidative stress–related genetic variants and advanced prostate cancer risk. *CAT* rs1001179 was associated with both stage III/IV and stage IV prostate cancer risk, with HRs per minor A allele of 1.16 [95% confidence intervals (CI), 1.01–1.33; P_{trend} = 0.032) and 1.25 (95% CI, 1.07–1.46; P_{trend} = 0.006), respectively. No other SNPs were associated with advanced prostate cancer risk.

Table 1. Baseline characteristics of advanced prostate cancer cases and subcohort members, Netherlands Cohort Study, 1986–2003

	Advanced (stage III/IV) prostate cancer[a] (*n* = 952)		Stage IV prostate cancer[b] (*n* = 595)		Subcohort members (*n* = 1,798)	
	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)
Age at baseline, y		62.1 (4.1)		62.3 (4.1)		61.3 (4.2)
Age at diagnosis, y		72.3 (5.3)		72.3 (5.3)		—
First-degree family history of prostate cancer	3.6		3.4		2.4	
Smoking status						
Never	12.8		13.1		12.6	
Former	52.9		55.2		52.3	
Current	34.3		31.7		35.0	
Duration of smoking, y[c]		32.4 (12.2)		33.1 (12.1)		32.8 (11.9)
Frequency of smoking, cigarettes/d[c]		16.1 (10.5)		16.4 (10.9)		16.9 (10.6)
High (>90 minutes/d) nonoccupational physical activity	35.1		33.0		31.8	
BMI, kg/m^2		25.0 (2.5)		25.1 (2.5)		25.0 (2.7)
History of diabetes	2.2		2.1		3.5	
Higher vocational or university degree	19.4		21.1		19.1	
Dietary intake						
Energy, kcal/d		2,143 (494)		2,126 (475)		2,145 (499)
β-carotene, mg/d		2.9 (1.4)		3.0 (1.5)		3.0 (1.6)
Catechin, mg/d		57.3 (37.9)		55.6 (36.9)		57.0 (37.1)
Lycopene, mg/d		1.2 (2.6)		1.2 (3.1)		1.1 (1.7)
Vitamin C, mg/d		100.2 (42.4)		100.2 (42.5)		98.2 (43.0)
Vitamin E, mg/d		15.0 (6.7)		14.8 (6.5)		14.6 (6.5)
Heme iron, mg/d		2.4 (1.0)		2.4 (1.1)		2.4 (1.0)
Alcohol, g/d		14.8 (16.0)		14.3 (11.5)		15.2 (16.6)
Toenail selenium, μg/g[d]		0.527 (0.174)		0.525 (0.203)		0.547 (0.113)

[a]Advanced (International Union Against Cancer, stage III/IV) prostate cancer was stage T3−4, N+, or M1 at diagnosis.
[b]Stage IV prostate cancer was stage T4, N+, or M1 at diagnosis.
[c]Never smokers were excluded.
[d]Eight hundred and seventeen stage III/IV prostate cancer cases (including 516 stage IV prostate cancer cases) and 1,048 subcohort had data on toenail selenium concentrations. A smaller version of the subcohort (70%) was used for this analysis.

Table 2. HR and 95% CIs for the association between oxidative stress–related genetic variants and advanced prostate cancer risk, Netherlands Cohort Study, 1986–2003[a]

			Advanced (stage III/IV) prostate cancer[b]			Stage IV prostate cancer[c]		
SNP rsID	**Genotype**	**Person-years**	**No. of events**	**HR (95% CI)**	**P_{trend}[d]**	**No. of events**	**HR (95% CI)**	**P_{trend}[d]**
CAT rs1001179	GG	15,794	552	1.00		335	1.00	
	AG	8,108	328	1.15 (0.96–1.36)		211	1.21 (0.99–1.49)	
	AA	1,282	60	1.38 (0.97–1.97)		43	1.64 (1.10–2.44)	
	Per minor allele	25,184	940	1.16 (1.01–1.33)	0.032	589	1.25 (1.07–1.46)	0.006
hOGG1 rs1052133	CC	15,170	546	1.00		341	1.00	
	CG	9,134	357	1.09 (0.92–1.29)		225	1.10 (0.90–1.34)	
	GG	1,122	49	1.25 (0.85–1.84)		29	1.19 (0.76–1.88)	
	Per minor allele	25,426	952	1.10 (0.96–1.27)	0.177	595	1.10 (0.93–1.29)	0.265
NOS2A rs2297518	GG	16,680	616	1.00		404	1.00	
	AG	7,724	311	1.09 (0.91–1.30)		175	0.93 (0.76–1.15)	
	AA	1,022	25	0.67 (0.42–1.08)		16	0.66 (0.37–1.15)	
	Per minor allele	25,426	952	0.99 (0.86–1.13)	0.839	595	0.89 (0.75–1.06)	0.179
NOS2A rs9282801	GG	10,370	407	1.00		266	1.00	
	GT	11,768	427	0.94 (0.79–1.12)		254	0.86 (0.70–1.05)	
	TT	3,288	118	0.95 (0.73–1.24)		75	0.93 (0.69–1.26)	
	Per minor allele	25,426	952	0.97 (0.86–1.09)	0.571	595	0.93 (0.81–1.08)	0.330
NOS3A rs1799983	GG	11,836	433	1.00		266	1.00	
	GT	11,147	418	1.02 (0.86–1.21)		269	1.07 (0.88–1.31)	
	TT	2,426	100	1.13 (0.85–1.49)		59	1.08 (0.77–1.51)	
	Per minor allele	25,409	951	1.05 (0.92–1.19)	0.468	594	1.05 (0.91–1.22)	0.493
NQ1 rs1800566	CC	17,901	662	1.00		413	1.00	
	CT	6,735	256	1.06 (0.88–1.27)		159	1.06 (0.85–1.31)	
	TT	778	34	1.16 (0.74–1.81)		23	1.25 (0.75–2.07)	
	Per minor allele	25,413	952	1.07 (0.92–1.24)	0.410	595	1.08 (0.91–1.29)	0.389
PON1 rs662	AA	12,938	481	1.00		313	1.00	
	AG	10,312	383	0.98 (0.83–1.16)		229	0.90 (0.74–1.10)	
	GG	2,176	88	1.08 (0.81–1.44)		53	1.00 (0.71–1.40)	
	Per minor allele	25,426	952	1.02 (0.90–1.15)	0.815	595	0.96 (0.82–1.11)	0.566
PON1 rs854560	AA	10,200	382	1.00		238	1.00	
	AT	11,914	444	0.99 (0.84–1.18)		272	0.98 (0.80–1.20)	
	TT	3,312	126	1.00 (0.78–1.30)		85	1.08 (0.81–1.45)	
	Per minor allele	25,426	952	1.00 (0.89–1.13)	0.991	595	1.02 (0.89–1.18)	0.735
SOD1 rs10432782	TT	19,595	732	1.00		446	1.00	
	GT	5,369	207	1.03 (0.84–1.25)		139	1.13 (0.90–1.42)	
	GG	461	13	0.78 (0.40–1.52)		10	0.99 (0.47–2.09)	
	Per minor allele	25,426	952	0.99 (0.83–1.17)	0.898	595	1.09 (0.90–1.33)	0.376
SOD2 rs4816407	AA	23,001	848	1.00		526	1.00	
	AG + GG	2,409	104	1.16 (0.89–1.51)		69	1.24 (0.91–1.68)	
SOD2 rs4880	TT	6,674	247	1.00		164	1.00	
	CT	12,605	479	0.99 (0.82–1.21)		294	0.91 (0.73–1.15)	
	CC	6,147	226	0.95 (0.75–1.20)		137	0.86 (0.66–1.13)	
	Per minor allele	25,426	952	0.98 (0.87–1.09)	0.666	595	0.93 (0.81–1.06)	0.283

Abbreviation: rsID, reference SNP ID number.

[a]All models were adjusted for age at baseline (y).

[b]Advanced (International Union Against Cancer, stage III/IV) prostate cancer was stage T3−4, N+, or M1 at diagnosis.

[c]Stage IV prostate cancer was stage T4, N+, or M1 at diagnosis.

[d]Analysis for linear trend according to the number of variant alleles (0, 1, or 2). All statistical tests were two sided.

Table 3 shows the statistical interactions between oxidative stress–related genetic variants and pro- and antioxidant intake and status on advanced (stage III/IV and IV) prostate cancer risk. We tested 151 SNP–environment interactions in relation to both stage III/IV and IV prostate cancer risk and identified 15 statistically significant multiplicative interactions (stage III/IV prostate cancer, $n = 8$; stage IV prostate cancer, $n = 7$). Seven of these interactions met the FDR Q-value <0.20 criterion; for stage III/IV prostate cancer, these involved intake of β-carotene (*GPX1* rs17650792, *hOGG1* rs1052133) and heme iron (*GPX1* rs1800668 and rs3448), and for stage IV prostate cancer, these involved intake of catechin (*SOD2* rs4880) and heme iron (*hOGG1* rs1052133, *SOD1* rs10432782). Stratified analyses using a single reference category (common homozygote genotype and lowest quartile of intake or status) showed: lowered risk for men with the AA genotype of *GPX1* rs17650792 in the third quartile of β-carotene intake (HR = 0.61; 95% CI, 0.40–0.93); elevated risk for men carrying the G allele of *hOGG1* rs1052133 in the first (HR = 1.49; 95% CI, 1.04–2.12) and third quartile of β-carotene intake (HR = 1.51; 95% CI, 1.08–2.11); lowered risk for men with the AA genotype of *GPX1* rs1800668 in the third quartile of heme iron intake (HR = 0.53; 95% CI, 0.34–0.82); elevated risk for men carrying the G allele of *hOGG1* rs1052133 in the first (HR = 1.48; 95%

Table 3. HR and 95% CIs for advanced prostate cancer risk according to pro- and antioxidant intake and status and oxidative stress–related genetic variants, Netherlands Cohort Study, 1986–2003[a]

Variable	Gene	SNP rsID	Genotype	No. of cases	Pro- and antioxidant intake or status					$P_{interaction}$[b]	FDR Q[c]
					HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)		
Advanced (stage III/IV) prostate cancer[d]											
Toenail selenium level					Q1	Q2	Q3	Q4			
	SOD2	rs4880	TT	205	1.00	0.87 (0.52–1.47)	0.83 (0.49–1.42)	0.75 (0.44–1.29)			
			CT + CC	612	1.40 (0.92–2.13)	1.03 (0.67–1.59)	0.61 (0.40–0.95)	0.44 (0.28–0.69)		0.025	0.28
β-carotene intake					Q1	Q2	Q3	Q4			
	GPX1	rs17650792	AA	282	1.00	0.81 (0.54–1.22)	0.61 (0.40–0.93)	0.86 (0.57–1.30)			
			AG + GG	621	0.84 (0.59–1.21)	1.00 (0.70–1.42)	1.10 (0.77–1.57)	0.85 (0.59–1.21)		0.021	0.10
β-carotene intake					Q1	Q2	Q3	Q4			
	hOGG1	rs1052133	CC	524	1.00	1.32 (0.98–1.79)	0.98 (0.72–1.34)	1.26 (0.93–1.72)			
			CG + GG	379	1.49 (1.04–2.12)	1.07 (0.77–1.50)	1.51 (1.08–2.11)	0.93 (0.66–1.30)		0.002	0.02
Vitamin C intake					Q1	Q2	Q3	Q4			
	NOS2A	rs9282801	GG	384	1.00	0.98 (0.68–1.41)	0.71 (0.49–1.02)	0.93 (0.64–1.34)			
			GT + TT	519	0.70 (0.49–0.99)	0.82 (0.58–1.14)	1.01 (0.72–1.42)	0.91 (0.65–1.27)		0.028	0.39
Vitamin C intake					Q1	Q2	Q3	Q4			
	SOD1	rs10432782	TT	693	1.00	1.14 (0.87–1.50)	1.29 (0.98–1.70)	1.24 (0.94–1.62)			
			GT + GG	210	1.53 (1.03–2.29)	1.38 (0.92–2.08)	0.88 (0.59–1.31)	1.24 (0.84–1.85)		0.035	0.25
Heme iron intake					Q1	Q2	Q3	Q4			
	GPX1	rs1800668	AA	282	1.00	1.18 (0.79–1.76)	0.53 (0.34–0.82)	0.82 (0.55–1.22)			
			AG + GG	621	0.95 (0.67–1.34)	1.03 (0.73–1.45)	1.01 (0.72–1.44)	1.08 (0.76–1.53)		0.014	0.19
Heme iron intake					Q1	Q2	Q3	Q4			
	GPX1	rs3448	CC	463	1.00	1.04 (0.75–1.44)	0.94 (0.66–1.32)	1.35 (0.97–1.88)			
			CT + TT	439	0.92 (0.66–1.27)	1.10 (0.80–1.52)	0.76 (0.54–1.05)	0.72 (0.52–1.01)		0.028	0.19
Alcohol intake					0 g/d	0.1–4 g/d	5–14 g/d	15–29 g/d	≥30 g/d		
	hOGG1	rs1052133	CC	519	1.00	1.37 (0.91–2.07)	1.67 (1.13–2.49)	1.11 (0.73–1.68)	1.10 (0.71–1.69)		
			CG + GG	375	1.41 (0.88–2.25)	1.42 (0.91–2.22)	1.19 (0.78–1.83)	1.40 (0.91–2.16)	1.65 (1.01–2.71)	0.037	0.52
Stage IV prostate cancer[e]											
Catechin intake					Q1	Q2	Q3	Q4			
	GPX1	rs17650792	AA	172	1.00	0.55 (0.34–0.88)	0.56 (0.35–0.90)	0.38 (0.23–0.64)			
			AG + GG	391	0.79 (0.53–1.17)	0.70 (0.47–1.05)	0.69 (0.46–1.02)	0.72 (0.49–1.08)		0.042	0.29
Catechin intake					Q1	Q2	Q3	Q4			
	SOD2	rs4880	TT	153	1.00	1.51 (0.91–2.51)	0.75 (0.43–1.30)	1.05 (0.61–1.80)			
			CT + CC	410	1.37 (0.89–2.12)	0.77 (0.49–1.21)	1.02 (0.66–1.57)	0.84 (0.54–1.32)		0.004	0.05
Heme iron intake					Q1	Q2	Q3	Q4			
	GPX1	rs17650792	AA	172	1.00	1.47 (0.92–2.38)	0.55 (0.32–0.96)	1.11 (0.69–1.79)			
			AG + GG	391	1.20 (0.79–1.82)	1.26 (0.83–1.90)	1.22 (0.80–1.86)	1.25 (0.82–1.91)		0.033	0.23
Heme iron intake					Q1	Q2	Q3	Q4			
	hOGG1	rs1052133	CC	326	1.00	1.22 (0.84–1.75)	1.10 (0.76–1.61)	1.40 (0.98–2.01)			
			CG + GG	237	1.48 (1.00–2.18)	1.66 (1.12–2.46)	0.92 (0.60–1.41)	1.05 (0.69–1.60)		0.035	0.16
Heme iron intake					Q1	Q2	Q3	Q4			
	SOD1	rs10432782	TT	420	1.00	1.35 (0.98–1.84)	1.15 (0.83–1.60)	1.24 (0.90–1.70)			
			GT + GG	143	2.14 (1.36–3.34)	1.61 (1.03–2.51)	0.73 (0.44–1.21)	1.37 (0.87–2.17)		0.006	0.09
Alcohol intake					0 g/d	0.1–4 g/d	5–14 g/d	15–29 g/d	≥30 g/d		
	hOGG1	rs1052133	CC	323	1.00	1.49 (0.92–2.43)	1.62 (1.01–2.60)	1.25 (0.77–2.04)	1.01 (0.60–1.71)		
			CG + GG	233	1.60 (0.92–2.77)	1.51 (0.89–2.56)	1.20 (0.72–2.01)	1.20 (0.71–2.03)	1.92 (1.09–3.40)	0.034	0.47
Oxidative balance score[f]					Q1	Q2	Q3	Q4			
	PON1	rs854560	AA	221	1.00	1.92 (1.21–3.05)	1.54 (0.98–2.42)	1.49 (0.93–2.37)			
			AT + TT	335	1.71 (1.11–2.62)	1.38 (0.90–2.11)	1.44 (0.94–2.21)	1.48 (0.97–2.28)		0.030	0.47

Abbreviations: rsID, reference SNP ID number; Q1–4, quartile 1 to 4.

[a]All models were adjusted for age at baseline (years). This table presents those interactions with a *P* for multiplicative interaction <0.05 ($n = 15$). The total number of gene–environment interaction tests was 302.

[b]The Wald statistic was used to test for multiplicative interaction. All statistical tests were two sided.

[c]FDR *Q* values were calculated for each pro- and antioxidant-specific interaction accounting for the number of SNPs investigated.

[d]Advanced (International Union Against Cancer, stage III/IV) prostate cancer was stage T3–4, N+, or M1 at diagnosis.

[e]Stage IV prostate cancer was stage T4, N+, or M1 at diagnosis.

[f]Sum of quartile or category scores (range from 0 to 3) of intake of β-carotene, catechin, lycopene, vitamin C, and vitamin E (all are antioxidants; high intake equals high score); and heme iron intake, smoking status (never, former, current), and alcohol intake (g/d; 0.1–4, 5–14, 15–29, ≥30; all are pro-oxidants; low intake equals high score). All score constituents had an equal weight.

CI, 1.00–2.18) and second quartile of heme iron intake (HR = 1.66; 95% CI, 1.12–2.46); and elevated risk for men carrying the G allele of *SOD1* rs10432782 in the first (HR = 2.14; 95% CI, 1.36–3.34) and second quartile of heme iron intake (HR = 1.61; 95% CI, 1.03–2.51). Of all 15 interactions, two displayed a decrease in risk over exposure categories. The first interaction was between *SOD2* rs4880 and toenail selenium level on advanced prostate cancer risk ($P_{interaction} = 0.025$; FDR $Q = 0.28$); compared with men with the common TT genotype in the lowest selenium quartile, carriers of the minor C allele (CT + CC) in the highest selenium quartile had an HR of 0.44 (95% CI, 0.28–0.69). The second interaction was between *GPX1* rs17650792 and catechin intake on stage IV prostate cancer risk ($P_{interaction} = 0.042$; FDR $Q = 0.29$); among men

with the common homozygote AA genotype those in the highest versus lowest quartile had an HR of 0.38 (95% CI, 0.23–0.64).

Discussion

In this large prospective study, we evaluated oxidative stress–related genetic variants in relation to advanced prostate cancer risk and examined potential interactions with pro- and antioxidant intake and status. The study showed an association between *CAT* rs1001179 and advanced prostate cancer risk. Marginal associations were not observed for any of the other tested SNPs. Of the observed 15 multiplicative gene–environment interactions, seven retained significance after multiple comparison adjustment using an FDR *Q*-value threshold of 0.20. These interactions were on either stage III/IV or stage IV prostate cancer risk and involved intake of β-carotene (*GPX1*, *hOGG1*), catechin (*SOD2*), and heme iron (*GPX1*, *hOGG1*, *SOD1*).

Catalase is an endogenous antioxidant enzyme that neutralizes hydrogen peroxide, a type of ROS, by converting it to water and oxygen (4). The enzyme plays an integral role in antioxidant defense against oxidative stress. *CAT* rs1001179 (A/G; also known as -262 C/T) is a common polymorphism located in the promoter region of the gene (49). A number of studies investigated associations between *CAT* rs1001179 and risk of cancer but mostly reported null findings (26). Only few studies investigated this SNP in relation to prostate cancer risk. In a nested case–control study (533 cases and 1,470 controls), Choi and colleagues showed no association between the SNP and overall prostate cancer risk (36). Recently, however, a small case–control study of prostate cancer in Turkey (155 cases and 195 controls) showed that *CAT* rs1001179 was associated with overall prostate cancer risk; men with the homozygote rare genotype compared with the homozygote common genotype had an HR of 1.57 (95% CI, 1.09–1.71; ref. 50). The authors also showed that among prostate cancer cases, the homozygote rare genotype was associated with advanced stage disease. Our analysis provides evidence of a role for *CAT* rs1001179 in prostate cancer by showing that the rare allele of the SNP was associated with increased risk of advanced prostate cancer. This finding is biologically plausible because the rare allele of *CAT* rs1001179 has been associated with lower catalase activity (51). A reduced catalase activity may result in deficient antioxidant protection against oxidative stress, which could potentially translate into a higher risk of developing prostate cancer. Our finding, however, requires replication from other observational studies including a large number of advanced prostate cancer cases.

We found no evidence of a marginal association for the ten other SNPs investigated. This is striking because we selected candidate SNPs that have been associated with prostate cancer risk in previous studies. The different outcome may result in part from the fact that we investigated advanced prostate cancer and the prior studies mostly investigated overall prostate cancer.

The significant gene–environment interactions (on stage III/IV or IV prostate cancer) in our study involved intake of β-carotene (*GPX1* rs17650792, *hOGG1* rs1052133), catechin (*SOD2* rs4880), and heme iron (*GPX1* rs1800668 and rs3448, *hOGG1* rs1052133, *SOD1* rs10432782). These interactions, which have not been reported before, met the FDR *Q*-value <0.20 criterion. The risk pattern of the interactions was unclear and could not be interpreted in terms of a dose–response relationship. We observed a significant altered risks for men in the third quartile of β-carotene and heme iron intake (*GPX1*, *hOGG1*) and evidence of a genotype effect (*hOGG1*, *SOD1*) for men with low heme iron intake. None of the statistical interactions showed a dose–response pattern that involved a distinct decrease or increase in risk overexposure categories. A number of epidemiologic studies investigated associations of these nutrients (intake or levels) with prostate cancer risk; and they generally do not support an association for β-carotene or heme iron (8, 16–18). Although our group previously showed that higher catechin intake was associated with lower risk of advanced prostate cancer (42), few other studies investigated the relationship and they generally do not support an association (42). Given the limited evidence of an association between these dietary nutrients and (advanced) prostate cancer risk, as well as that few other studies comprehensively evaluated oxidative stress–related genetic variation in relation to pro- and antioxidant exposure and prostate cancer risk, the positive results in this study require replication.

Although the seven statistically significant interactions did not involve a clear dose–response pattern, two other multiplicative interactions (P <0.05), that were however not significant after multiple testing correction (FDR *Q*-value >0.20), did involve a dose–response pattern. The risk pattern showed a decrease in risk over antioxidant exposure categories in one genotype subgroup but not the other. The first interaction was between selenium levels and *SOD2* rs4880 on stage III/IV prostate cancer risk and involved a 56% lower risk for men carrying the C allele in the highest quartile of selenium. This interaction has been reported previously in a nested case–control study in the Physicians' Health Study, showing a similar risk pattern as in our study (28). The interaction is supported by mechanistic data and possibly results from an enzyme imbalance between SOD2 and GPX, which is selenium dependent and removes hydrogen peroxide produced by SOD2 (28). The second interaction was between catechin intake and *GPX1* rs17650792 on stage IV prostate cancer risk and involved a 62% lower risk for men with the homozygote AA genotype in the highest quartile of intake. As far as we know, there is no mechanistic data supporting this novel interaction.

Oxidative stress has been hypothesized to play an important role in prostate cancer (6, 7). To shed additional light on this link, we searched for gene–environment interactions between well-known oxidative stress–related gene variants and major pro- and antioxidant exposures. This type of hypothesis-driven pathway analysis is important to discover gene–environment interactions. Investigating interactions in gene-association studies with larger numbers of SNPs, including GWAS, remains challenging because of the very large number of tests required. The most effective way to apply and detect interactions in the context of GWAS remains unresolved (52).

The most important strengths of our study include its prospective design, population-based approach, and long and nearly complete follow-up of the study population through linkage to cancer registries. We specifically evaluated advanced stage prostate cancer because these cancers have a poor prognosis and are therefore clinically relevant (29). Intake of selenium cannot be accurately estimated via an FFQ because of variations in the selenium content of soil and concomitant variability in the selenium content of foods (10). We therefore used toenail selenium for exposure monitoring, which reflects long-term selenium intake (10).

Our study has some limitations. First, measurement of dietary intake using questionnaires may result in misclassification and potentially attenuated risk estimates, even though the study participants under study (ages 55 to 69 years) had relatively stable diets. Furthermore, we used a baseline exposure measurement only in combination with long-term follow-up for cancer incidence (17.3 years). Second, for most genes in our study, we only selected one or two SNPs and we may therefore have potentially missed important SNPs that modify advanced prostate cancer risk.

In conclusion, this large prospective study on advanced prostate cancer risk showed an association with a *CAT* polymorphism and identified seven novel statistically significant gene–environment interactions. Additional well-powered gene-association studies are needed to confirm these findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The study sponsor had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

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Acknowledgments

The authors thank the study participants, the Netherlands Cancer Registry, the Dutch Pathology Registry, and the Biobank of the Maastricht University Medical Center. We also thank Sacha van de Crommert, Dr. Leo Schouten, Jolanda Nelissen, Conny de Zwart, Ellen Dutman, Henny Brants, and Annemie Pisters for their assistance with data entry or data management, Harry van Montfort for programming assistance, and Stijn Lumeij, Kristien Lemmens, Joy Goessens, Leonie Jonkers, Roger Godschalk, and Simone van Breda for technical assistance with DNA isolation and genotyping.

Grant Support

This work was supported by a research grant from the Dutch Cancer Society (grant number: UM 2009-4556).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 19, 2014; revised October 8, 2014; accepted October 8, 2014; published OnlineFirst October 14, 2014.

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Oxidative Stress–Related Genetic Variants, Pro- and Antioxidant Intake and Status, and Advanced Prostate Cancer Risk

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Cancer Epidemiol Biomarkers Prev 2015;24:178-186. Published OnlineFirst October 14, 2014.