Research Article

Cancer Epidemiology, Biomarkers & Prevention

Effects of Selenium Status and Polymorphisms in Selenoprotein Genes on Prostate Cancer Risk in a Prospective Study of European Men

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Abstract

Background: Evidence for an association between selenium status and prostate cancer risk is still inconclusive. Anticarcinogenic effects of selenium are supposedly mediated through cellular protective and redox properties of selenoenzymes *in vivo*. We evaluated the association between serum selenium status and prostate cancer risk in a population with relative low selenium concentrations considering effect modification by genetic variants in selenoprotein genes.

Materials and Methods: A case-control study of 248 incident prostate cancer cases and 492 matched controls was nested within the EPIC-Heidelberg cohort. Baseline blood samples were analyzed for serum selenium and selenoprotein P concentrations and glutathione peroxidase activity. Genotyping was carried out for *SEP15* (rs5859, rs540049), *SEPP1* (rs3877899, rs7579), *GPX1* (rs1050450), and *GPX4* (rs713041). Conditional logistic regression was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (95% CI).

Results: The OR for prostate cancer was 0.89 (95% CI, 0.79-1.01) per 10 μg/L increase of serum selenium concentration. This association was modified by rs1050450 (C>T) in *GPX1* ($P_{interaction}$ = 0.03), with carriers of one or two T alleles having a significantly reduced OR of 0.87 (95% CI, 0.76-0.99). Furthermore, there was an association between rs7579 genotype in *SEPP1* and prostate cancer risk (OR, 1.72; 95% CI, 0.99-2.98).

Conclusions: Our results support a role of selenium and polymorphisms in selenoenzymes in prostate cancer etiology, which warrants confirmation in future studies.

Impact: These findings might help to explain biological effects of selenium in prostate cancer development in order to overcome inconsistencies arising from former studies. *Cancer Epidemiol Biomarkers Prev; 19(11); 2958–68. ©2010 AACR.*

Introduction

Selenium (Se) is a trace element essential for human health. It is specifically incorporated as the amino acid selenocysteine into 25 so-called selenoproteins, many of which catalyze redox reactions with Se at the active site (1-3). Increased intake of Se has been suggested to have anticarcinogenic effects, and numerous mechanisms have been proposed to explain this property: reduction of DNA damage, oxidative stress, or inflammation; also, induction of phase II enzymes, enhancement of immune response, inhibition of cell cycle and angiogenesis, and induction of apoptosis were proposed (4). Intervention studies in humans have shown that supplementation of Se leads to an increase in concentration and/or activity of circulating selenoproteins, such as selenoprotein P (SePP) or glutathione peroxidases (GPx; refs. 1, 5), and plasma SePP and GPx3 represent common markers of Se status. Because GPx are important components of the redox control system in humans, reduction of cellular oxidative stress and subsequent DNA damage could be a major mechanism to explain the anticancer effects of Se. It is likely that the anticarcinogenic properties of Se *in vivo* are mediated by specific selenoproteins in the target organ.

A protective effect of Se in prostate cancer development was originally suggested by a Se supplementation trial carried out in the United States, the Nutritional Prevention of Cancer (NPC) trial (6). However, recent results from the SELECT trial do not support this conclusion (7). These conflicting observations may reflect differences in baseline plasma Se status at the start of the two trials. In the NPC trial, the beneficial effects of Se regarding prostate cancer incidence were observed in subjects with

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doi: 10.1158/1055-9965.EPI-10-0364

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a low plasma Se concentration at baseline (8), whereas in the SELECT study baseline plasma Se was higher than this low level and participants were not stratified according to Se status. In Europe, a relatively low Se status comparable with that in the bottom tertile of the NPC study is common (9), suggesting that Se supplementation could elicit some prostate cancer protection in European men. In addition, recent data not only indicate that genetic variations in selenoprotein genes account for interindividual differences in response to Se supplementation (10, 11) but also that they may influence cancer risk (12, 13). Such observations may help to reconcile the complex observations from both the NPC and SELECT studies and also identify subgroups of the population at increased or decreased risk of prostate cancer depending on their Se status and capacity to respond to Se intake.

The aim of the present study was to investigate the association between Se status (as assessed by measurement of total serum Se, SePP concentration, and serum GPx3 activity) and prostate cancer risk in a European population with an average Se status considerably lower than that in the United States and to evaluate possible effect modification by single-nucleotide polymorphisms (SNP) in selected genes. These genes either encode for selenoproteins, which are highly expressed in the prostate (Sep15; ref. 14) and/or possess functions in Se transport (SePP) or protection from oxidative and endoplasmic reticulum stress (GPx1, GPx4, Sep15); we also focused on specific variants for which there is evidence of functional consequences and possible links to either Se status or cancer risk (10, 11, 14-17). Thus, our hypothesis is that selenoprotein functions within the prostate gland influence prostate cancer risk and that these functions are affected by both Se intake and genetic variation in the ability to transport Se to the prostate and Se-dependent cellular protection mechanisms.

Materials and Methods

Study population and data assessment

EPIC-Heidelberg was designed as a prospective cohort study aiming to evaluate the association between dietary, lifestyle, and metabolic factors and the risk of cancer. A random sample of the general population of Heidelberg, Germany, and surrounding communities was provided by the local registries and invited to participate. From 1994 to 1998, 11,928 men (ages 40-64) and 13,612 women (ages 35-64) were recruited, comprising 38% of those approached (18). During a baseline visit at the examination center, dietary, lifestyle, medical, and socioeconomic data were collected via self-administered questionnaires and personal interview. Questions addressed occupational situation, education, socioeconomic status, physical activity at work and during leisure time, lifetime consumption of alcohol and tobacco, and medical history. Anthropometric measures including weight, height, waist, and hip circumferences were taken by trained personnel following standardized procedures (19). Additionally, blood samples were taken from 95.8% of the participants; these were fractionated into serum, plasma, buffy coat, and erythrocytes and subsequently stored in liquid nitrogen at −196°C. All participants gave written informed consent, and the study was approved by the ethics committee of Heidelberg Medical School.

Every 2 to 3 years, participants were contacted by follow-up questionnaires to assess information on health status. Participation rates of the completed three follow-ups were >90%. Medical records or death certificates were examined to verify self-reported cases of prostate cancer (C61, C63.8, and C63.9; International Classification of Diseases for Oncology, 2nd edition). Additionally, information on tumor grade (Gleason histologic grade) was assembled and used to categorize cases as high-grade (Gleason score ≥ 7), low grade (<7), or unknown. Based on all male EPIC-Heidelberg participants with blood samples available and free of prevalent cancer at baseline (except nonmelanoma skin cancer), we set up a nested case-control study. Incident prostate cancer cases diagnosed by the end of February 2007 were selected, and two controls were matched per case by age (5-year age groups) and time of recruitment (6-month intervals) following an incidence density matching protocol (20). The final nested case-control study comprised 248 cases and 492 controls. During the second and third follow-up rounds, questions addressed history of prostate cancer in first-degree relatives and participation in prostate-specific antigen (PSA) screening. Only those cases who participated in screening before the date of cancer diagnosis were coded as having a positive screening history. Similarly, only controls participating in screening before the date of diagnosis of the index case were classified as controls participating in prostate cancer screening.

Measure of GPx3 activity, serum Se, and serum SePP concentration

GPx activity was determined with Ransel RS 505 kits (Randox) based on the UV method by Paglia and Valentine (21). Slight alterations of the manufacturer's protocol were undertaken to account for using serum instead of whole blood. Absorbance readings on the LX-20 Pro autoanalyzer (Beckman) were done between 90 and 260 seconds at 340 nm with 380 nm as reference. The intraassay coefficient of variation was 2.7%. The interassay coefficients of variation for the four measurement days lay between 1.3% and 2.4%. No sample was under the detection limit of 20 units/L, and none exceeded the upper limit of 3,500 units/L. Blinded quality control samples were not.

Total serum Se was determined by dynamic reaction cell-inductively coupled plasma field mass spectrometry at Southampton University Hospital as described by Sieniawska et al. (22) on Elan 6100 DRC plus (SCIEX Perkin-Elmer). The detection limit was set at 0.02 μmol/L (1.58 μg/L). In each assay, four quality control samples were included, and the interassay coefficient of variation ranged from 3.0% to 6.2% whereas the intraassay coefficient of

variation lay between 2.9% and 4.4%. Six samples had insufficient amounts of serum to be analyzed. No blinded quality control samples were used. Average measurement of reference samples (Nycomed) were within the desirable range (0.87 ± 0.05 and 1.77 ± 0.07 μmol/L, with corresponding target values of 0.92 and 1.72 μmol/L).

SePP concentration was measured by an immunoluminometric sandwich assay (23). Chemiluminescence was measured for 1 second with a luminometer LB952T (Berthold). Based on five standards of known concentration, ranging from 0.13 to 10.65 mg SePP/L, that were measured in each assay run, a standard curve was fitted to the data. Each sample was measured in triplicate, and mean SePP concentration was calculated. Due to a loss of antigenicity of the standard solutions during storage, a random sample of serums covering each of the different measurement days was reanalyzed with new standard solutions in a separate assay. Based on these measurements, the concentrations of all samples were calibrated. For quality checks, two control samples were measured in each assay, and intraday and interassay coefficients of variation were <10% for SePP values of >0.15 mg/L. For two participants, there was not enough serum available for analysis. Reference samples with known concentrations for quality control were not available, and no blinded quality control samples were used in the analysis.

Genomic DNA was extracted from buffy coat with FlexiGene kit (Qiagen) in accordance with the manufacturer's instructions. DNA was stored at 4°C until use. Genotyping for polymorphisms of the genes *SEP15* (G1125A, rs5859), *SEP15* [C>T in 3′ untranslated region (UTR), rs540049], *SEPP1* (G>A coding, Ala234Thr, rs3877899), *SEPP1* (G>A in 3′ UTR, rs7579), *GPX1* (C>T, Pro198Leu, rs1050450), and *GPX4* (C718T, rs713041) were done as multiplex on the MassArray system (Sequenom) applying the iPLEX method and matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry for analyte detection. The analysis was carried out by Bioglobe. All duplicated samples (quality control repeats of 8% of the samples) to verify interexperimental reproducibility and accuracy delivered concordant genotype results. Similarly, a control sample applied on every plate yielded identical genotypes. The genotype could not be assigned to six samples for the *GPX1* C>T (rs1050450) SNP and to five samples for the *GPX4* C718T (rs713041) SNP. All laboratory analyses were carried out with the laboratory personnel blinded to the case-control status.

Statistical analysis

Baseline characteristics of the study population are given as mean and SD or percentages by case-control status. Serum Se and SePP concentrations as well as GPx3 activity were nearly normally distributed and are presented as mean and SD.

Among healthy controls, Pearson correlation coefficients were computed for serum Se and SePP concentration and GPx3 activity. Generalized linear models were used to compare mean serum Se concentration adjusted for matching variables over categories of lifestyle factors and genotypes of the selected selenoproteins, and *P* values for difference were calculated. Additionally, mean serum SePP concentration and GPx3 activity were calculated over genotypes. Genotype frequencies for the selected polymorphisms were computed, and deviations from Hardy-Weinberg equilibrium (HWE) were determined by χ^2 test. Conditional logistic regression stratified by matched case set was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the association of the SNPs with prostate cancer risk, using the carriers of the homozygous common allele as reference category. For the two SNPs in *SEP15*, only few subjects were carriers of the homozygous less frequent allele; thus, they were combined with those heterozygous.

Main effects of serum Se or SePP concentration or GPx3 activity on prostate cancer risk were calculated by conditional logistic regression stratified by case set using the continuous variables as well as quartiles based on the distribution among the controls. Adjustments were made for possible confounding factors including family history of prostate cancer in first-degree relatives (yes/no), participation in PSA screening (yes/no), smoking status (never/former/current), educational attainment (no or primary school/secondary or technical school/university degree), body mass index (BMI, continuously), or vigorous physical activity (none, ≤2 h/wk, >2 h/wk). The final model included participation in PSA screening, smoking status, vigorous physical activity, and family history of prostate cancer as adjustments, because inclusion of these factors changed the OR by >10% when comparing extreme quartiles. We repeated the analysis after excluding cases (and matched controls) diagnosed within the first year of follow-up and in the subgroups of high-grade and low-grade prostate cancer cases (and matched controls). We further evaluated potential effect modification by vitamin E intake, vitamin C intake, β-carotene intake, or smoking status. Additionally, we did spline regression to modulate a potential U-shaped association between serum Se concentration and prostate cancer with knots set at the cut points of the quartiles of Se status in controls.

To evaluate potential effect modification of the association between serum Se concentration and prostate cancer risk by genotype, we calculated OR (and 95% CI) of prostate cancer for the continuous Se variable stratified by genotype with unconditional logistic regression adjusting for the matching variables (time of recruitment and 5-year age group). Additionally, adjustments were made for family history of prostate cancer, participation in PSA screening, smoking status, and vigorous physical activity. Due to small numbers in the homozygous less frequent genotype, we also combined the heterozygote and homozygote less frequent categories. We tested for interaction by comparing the unconditional logistic regression model with and without cross-product terms (of genotype and continuous Se variable) based on the likelihood ratio statistic. This analysis was repeated in

the subgroup of high-grade prostate cancer cases and their matched controls.

All analyses were done with SAS 9.1 (SAS Institute).

Results

Baseline characteristics of the study population are presented in Table 1. Cases and controls did not differ by age (matching factor) or BMI. Cases were less likely to be former or current smokers and had less often a university degree. They were more likely to have participated in PSA testing, to engage in vigorous physical activity, and to drink >15 g alcohol per day. Additionally, more cases than controls reported a positive family history of prostate cancer. There seemed to be no meaningful differences in mean serum concentration of Se or SePP or GPx3 activity between cases and controls. Median time between blood collection and diagnosis of prostate cancer was 5.3 years (range, 0.01-10.6 years). Overall, based on Gleason histologic grade, 92 cases were classified as high-grade and 130 as low-grade tumors; for 26 cases, no information was available.

The association of serum Se concentration and prostate cancer risk is shown in Table 2. The OR for prostate cancer decreased across quartiles of serum Se concentration with a significantly reduced risk in the third quartile compared with the first quartile (adjusted OR, 0.61; 95% CI, 0.38-0.98; $P = 0.04$), but not in the fourth quartile. When modeling serum Se concentration as continuous variable, we found a reduced OR of 0.89 per 10 μg/L serum Se (95% CI, 0.79-1.01; $P = 0.07$) that was of borderline significance. Excluding cases (and matched controls) diagnosed within the first year of follow up did not alter these results. When we restricted our analysis to the subgroup of cases (case sets) with high-grade cancer, the effects were

Table 1. Baseline characteristics of cases and controls in the EPIC-Heidelberg nested case-control study ($n = 740$)

	Cases ($n = 248$)		Controls ($n = 492$)	
	Mean	**SD**	**Mean**	**SD**
Age (y)	58.1	±4.8	58.1	±4.8
BMI (kg/m^2)	27.3	±3.6	27.3	±3.4
	N	Percent	*N*	Percent
Smoking status				
Never	99	39.9	166	33.7
Former	112	45.2	241	49.0
Current	37	14.9	85	17.3
Educational attainment				
No/primary school	89	35.9	158	32.1
Secondary/technical school	82	33.1	153	31.1
University	77	31.1	181	36.8
Positive family history of prostate cancer	18	7.3	9	1.8
Participation in PSA screening*	58	23.4	88	17.9
Vigorous physical activity				
None	88	35.5	201	40.9
≤2 h/wk	79	31.9	163	33.1
>2 h/wk	81	32.7	128	26.0
Alcohol intake (g/d)				
<5	41	16.5	94	19.1
5-15	54	21.8	124	25.2
15-30	67	27.0	112	22.8
≥30	86	34.7	162	32.9

	N	Mean	SD	*N*	Mean	SD
Serum selenium (μg/L)	244	86.2	±14.2	490	87.7	±13.4
Serum SePP (mg/L)	247	2.88	±0.51	491	2.92	±0.52
Serum GPX (units/L)	248	647.6	±112.9	492	656.7	±110.2

*In the period before the index case was diagnosed with PCa.

Table 2. Association between serum selenium status and prostate cancer risk in the EPIC-Heidelberg nested case-control study (n = 740)

	Quartiles of serum selenium concentration (μg/L)				Continuous (per 10 μg/L)
	<78.9	78.9-87.0	87.0-95.0	≥95.0	
Cases/controls (n)	69/116	66/125	43/122	66/127	
Crude OR* (95% CI)	1.00	0.90 (0.58, 1.40)	0.60 (0.38, 0.95)	0.89 (0.58, 1.37)	0.92 (0.82, 1.04)
Adjusted OR† (95% CI)	1.00	0.92 (0.59, 1.43)	0.61 (0.38, 0.98)	0.78 (0.49, 1.22)	0.89 (0.79, 1.01)
Excluding cases diagnosed during 1st year of follow-up (and matched controls)					
Cases/controls (n)	68/112	59/116	40/117	64/119	
Adjusted OR† (95% CI)	1.00	0.87 (0.55, 1.37)	0.57 (0.35, 0.92)	0.79 (0.50, 1.25)	0.89 (0.78, 1.01)
High-grade prostate cancer					
Cases/controls (n)	31/37	21/46	12/41	26/56	
Adjusted OR† (95% CI)	1.00	0.53 (0.25, 1.12)	0.34 (0.15, 0.79)	0.50 (0.24, 1.03)	0.84 (0.69, 1.02)
Low-grade prostate cancer					
Cases/controls (n)	35/68	31/67	26/67	36/56	
Adjusted OR† (95% CI)	1.00	0.91 (0.48, 1.71)	0.75 (0.40, 1.41)	1.10 (0.58, 2.09)	0.96 (0.81, 1.15)

*Crude OR from conditional logistic regression stratified by matching factors (age group and time of recruitment).
†Adjusted OR additionally adjusted for family history of prostate cancer, participation in PSA testing, smoking status, and vigorous physical activity.

more pronounced. Similar results were obtained for high-stage tumors (data not shown). The association between Se concentration and prostate cancer risk was not modified by smoking status ($P_{interaction}$ = 0.59), tertile of vitamin C intake ($P_{interaction}$ = 0.84), tertile of vitamin E intake ($P_{interaction}$ = 0.66), or tertile of β-carotene intake ($P_{interaction}$ = 0.54; data not shown). We did restricted cubic spline regression with knots set at the cut points of the quartiles, but the test for nonlinearity was not statistically significant (P = 0.38). Using serum SePP concentration as continuous exposure variable, we observed a nonsignificant inverse association (adjusted OR, 0.77; 95% CI, 0.55-1.07; P = 0.12; per 1 mg/L serum SePP) with prostate cancer risk, whereas quartiles of SePP concentration were not associated with cancer risk [adjusted OR (95% CI): 0.83 (0.52-1.33), 1.07 (0.68-1.68), and 0.83 (0.51-1.33) for the second, third, and fourth quartile, respectively]. The adjusted OR for prostate cancer for an increase of 100 units/L GPx3 activity was 0.91 (95% CI, 0.79-1.04; P = 0.17), and the analysis in quartiles showed similar patterns as for serum Se with a significantly reduced OR in the third quartile compared with the first quartile (adjusted OR, 0.63; 95% CI, 0.39-0.99; P = 0.05) but not in the fourth quartile (adjusted OR, 0.85; 95% CI, 0.55-1.31; P = 0.46; see also Appendix Tables 1 and 2.

Genotype frequencies for cases and controls of the selected polymorphisms and associations with prostate cancer risk are shown in Table 3. Genotype frequencies of the selected SNPs in controls did not depart from HWE and were in accordance with those reported for Caucasians where available (10, 19-21). An increased risk of prostate cancer was observed in homozygous AA carriers for rs7579 in the *SEPP1* gene (OR, 1.72; 95% CI, 0.99-2.98; P = 0.05).

Within the group of healthy participants, statistically significant correlations of serum Se with SePP (Pearson correlation, r = 0.68, P < 0.001) concentrations or serum Se with serum GPx3 activity (r = 0.35, P < 0.001) were observed. The correlation between serum Se and GPx3 activity was significant (r = 0.32, P < 0.001) in participants with serum Se concentration of <92 μg/L, but it disappeared (r = 0.09; P = 0.22) in participants with a Se concentration of >92 μg/L (n = 175). This serum Se concentration is considered to represent the Se status required to maximize GPx3 activity (22). The correlation between serum SePP and GPx3 activity was 0.24 (P < 0.01). Serum Se concentration did not vary significantly by age, BMI group (BMI: < 25, 25–30, or ≥30 kg/m^2), educational attainment, smoking status, vigorous physical activity, alcohol intake category, participation in PSA screening, or family history of prostate cancer (data not shown).

Table 4 presents the mean concentration of serum Se, serum SePP concentration, and GPx3 activity in genotype strata of the selected SNPs in selenoprotein genes in the group of healthy participants. A main effect of genotype for rs7579 in the *SEPP1* gene was observed on serum SePP concentration (borderline statistical significance, P = 0.07). Combining heterozygous and homozygous carriers of the less frequent allele resulted in a significantly higher mean SePP serum concentration (P = 0.04) compared with carriers of the homozygous common allele. An effect of genotype for both SNPs in the *SEP15* gene (rs5859 and rs540049) was observed on the activity of serum GPx3 (P = 0.05) with an overall decreased activity of GPx3 in the rare homozygote genotype.

The effects of genotype for the selected polymorphisms in selenoprotein genes on the association between Se

concentration and prostate cancer risk by selected polymorphisms are shown in Table 5. Subjects with the less frequent T alleles (heterozygote and homozygote) for rs1050450 in *GPX1* had an OR of 0.87 (95% CI, 0.76-0.99; P = 0.04) per 10 μg/L increase in serum Se concentration, whereas in subjects homozygous for the common C allele, no association was found ($P_{\text{interaction}}$ = 0.03). When we restricted our analyses to high-grade prostate cancer, the risk ratio was even more reduced (OR, 0.64; 95% CI, 0.49-0.83; P = 0.001, $P_{\text{interaction}} < 0.001$). For other polymorphisms in selenoprotein genes, no effect modification of the serum selenium–prostate cancer association was found.

Discussion

Previous work has suggested a possible inverse association between Se levels and risk of prostate cancer (24). The results of the present study support this suggestion by showing a significantly decreased risk of prostate cancer, especially high-grade prostate cancer, in the third quartile of serum Se concentration, and extend it by showing a significant interaction between serum Se concentration and *GPX1* genotype rs1050450 with respect to high-grade prostate cancer. Furthermore, the data suggest that there is also an effect of genotype for rs7579 in *SEPP1* gene on prostate cancer risk.

Estimating Se intake using food questionnaire data is hampered by the high variation of Se content in the same food; therefore, it is preferable to use biomarkers such as serum selenium, SePP, or GPx3 to assess Se status (5, 25). Because each of the three biomarkers provide slightly different information, all three were analyzed in this study. The dose of Se required to optimize GPx3 and SePP is different, and it seems possible that tissue (prostate) will respond differently to the form of Se provided, possibly also depending on genetic variants in key enzymes. Among controls, there was fairly good correlation between several indicators of Se status in our study. The correlation of serum Se with GPx3 activity leveled off for Se concentrations of >92 μg/L, which is compatible with the earlier finding that this plasma Se concentration is needed to achieve full expression of plasma GPx3 activity (26). In addition, our observed correlation between serum Se and serum SePP is consistent with previous reports from European populations (27, 28); in general, the Se concentration in our healthy participants was in line

Table 3. Genotype frequencies of selected polymorphisms in selenoproteins and association with prostate cancer risk in the EPIC-Heidelberg nested case-control study

Gene SNP (rsNo.)	Genotype	Cases (%)	Controls (%)	HWE*	OR†	95% CI
SEP15	GG	165 (66.5)	309 (62.8)		1.00	
G1125A	GA	78 (31.5)	157 (31.9)		0.84	(0.60, 1.17)
(rs5859)	AA	5 (2.0)	26 (5.3)			
				0.31		
SEP15	CC	165 (66.5)	325 (66.1)		1.00	
C>T	CT	78 (31.5)	142 (28.9)		0.98	(0.70, 1.37)
(rs540049)	TT	5 (2.0)	25 (5.1)			
				0.07		
SEPP1	GG	152 (61.3)	271 (55.1)		1.00	
G>A coding	GA	86 (34.7)	194 (39.4)		0.80	(0.58, 1.10)
Ala234Thr	AA	10 (4.0)	27 (5.5)		0.66	(0.31, 1.39)
(rs3877899)				0.31		
SEPP1	GG	116 (46.8)	250 (50.8)		1.00	
G>A 3′UTR	GA	105 (42.3)	209 (42.5)		1.08	(0.78, 1.49)
(rs7579)	AA	27 (10.9)	33 (6.7)		1.72	(0.99, 2.98)
				0.22		
GPX1	CC	123 (49.8)	264 (54.2)		1.00	
C>T	CT	108 (43.7)	181 (37.2)		1.30	(0.95, 1.78)
Pro198Leu	TT	16 (6.5)	42 (8.6)		0.81	(0.44, 1.49)
(rs1050450)				0.18		
GPX4	CC	77 (31.4)	156 (31.8)		1.00	
C718T	CT	114 (46.5)	229 (46.7)		1.00	(0.70, 1.44)
(rs713041)	TT	54 (22.0)	105 (21.4)		1.03	(0.68, 1.57)
				0.22		

*P value of χ^2 test for deviation from HWE in controls.
†Conditional logistic regression stratified by matching factors (age group and time of blood collection).

Table 4. Mean and 95% confidence interval of serum selenium and SePP concentration and GPx3 activity over genotypes of SNPs in selected selenoproteins in healthy participants of the EPIC-Heidelberg nested case-control study

	N	Serum Se concentration (μg/L)*	*P*†	*N*	Serum SePP concentration (mg/L)*	*P*†	*N*	Serum GPx3 activity (units/L)*	*P*†
SEP15 G1125A (rs5859)									
GG	307	87.9 (86.4, 89.4)		308	2.91 (2.85, 2.96)		309	656.5 (644.4, 668.7)	
GA	157	87.7 (85.6, 89.8)		157	2.96 (2.88, 3.04)		157	665.2 (648.1, 682.2)	
AA	26	84.0 (78.9, 89.2)	0.36	26	2.83 (2.64, 3.03)	0.36	26	607.7 (565.8, 649.7)	0.05
SEP15 C>T (rs540049)									
CC	323	88.0 (86.5, 89.5)		324	2.91 (2.85, 2.96)		325	655.9 (644.1, 667.8)	
CT	142	87.5 (85.3, 89.7)		142	2.97 (2.88, 3.05)		142	666.9 (649.0, 684.9)	
TT	25	84.2 (78.9, 89.4)	0.38	25	2.83 (2.63, 3.04)	0.33	25	609.2 (566.4, 652.0)	0.05
SEPP1 G>A coding (rs3877899)									
GG	270	88.1 (86.5, 89.7)		271	2.95 (2.89, 3.01)		271	659.2 (646.1, 672.2)	
GA	193	87.5 (85.7, 89.4)		193	2.90 (2.82, 2.97)		194	652.5 (637.0, 667.9)	
AA	27	83.6 (78.6, 88.7)	0.25	27	2.83 (2.64, 3.03)	0.40	27	662.8 (621.4, 704.3)	0.77
SEPP1 G>A 3′UTR (rs7579)									
GG	250	87.1 (85.4, 88.7)		250	2.87 (2.81, 2.94)		250	651.8 (638.2, 665.4)	
GA	207	88.7 (86.8, 90.5)		208	2.98 (2.91, 3.05)		209	664.3 (649.4, 679.2)	
AA	33	85.8 (81.2, 90.4)	0.31	33	2.89 (2.71, 3.06)	0.07	33	645.9 (608.6, 683.3)	0.40
GPX1 C>T (rs1050450)									
CC	263	87.9 (86.2, 89.5)		264	2.95 (2.88, 3.01)		264	655.1 (641.8, 668.4)	
CT	180	87.7 (85.7, 89.6)		180	2.91 (2.84, 2.99)		181	661.0 (644.9, 677.0)	
TT	42	86.8 (82.7, 90.9)	0.89	42	2.82 (2.67, 2.98)	0.33	42	643.5 (610.1, 676.9)	0.63
GPX4 C718T (rs713041)									
CC	155	87.8 (85.7, 89.9)		155	2.97 (2.89, 3.05)		156	663.8 (646.6, 681.1)	
CT	228	87.1 (85.4, 88.8)		229	2.86 (2.80, 2.93)		229	651.2 (637.0, 665.5)	
TT	105	88.3 (85.8, 90.9)	0.71	105	2.97 (2.87, 3.06)	0.07	105	658.4 (637.4, 679.4)	0.54

*Values are mean and (95% CI) adjusted for matching factors (age group and time of blood collection).
†*P* values for difference derived from generalized linear models.

with those reported in other European populations but lower than those reported from studies in the USA, where Se supply of the population is distinctly higher (9). Similarly, the range of SePP concentration in our population matched those reported in another German (23) and Danish (29) studies.

We found an inverse association of borderline statistical significance between prediagnostic serum Se concentrations and total prostate cancer risk. This finding is in line with some other nested case-control studies measuring serum or plasma Se (30, 31) or toenail Se (32, 33) but not all (34-38). In a recent metaanalysis (24), 20 observational studies of different design (case-control, nested case-control, cohort, case-cohort), in which Se was measured in serum, plasma, or toenails, showed that men with low serum Se levels are at increased risk of prostate cancer. Our finding of a stronger effect of Se concentration in high-grade prostate cancer is in agreement with some (30, 32, 34, 39) but not all (36-38) studies.

One possible explanation of this somewhat inconsistent picture is that subjects with low Se levels might benefit most from increasing Se status. In the NPC Se supplementation trial, it has been shown that prostate cancer incidence in the lower two tertiles of baseline Se concentrations was reduced by supplementation, whereas supplementation in the highest tertile had no effect ($P_{interaction}$ = 0.02; ref. 40). Interestingly, our results based on quartiles of Se status showed a significantly decreased risk for the third quartile, but not for the fourth quartile, which comprised participants with a serum concentration of >95 μg/L. However, our spline analysis did not hint at a nonlinear relationship. Furthermore the Se status of the individuals in the present study was considerably lower than the median baseline Se status in the recent SELECT study (7), compatible with the suggestion that the lack of benefit of Se supplementation on prostate cancer incidence observed in SELECT may reflect the high baseline Se status of the individuals recruited into that trial.

The anticarcinogenic effects of Se may depend on the status of other antioxidants such as vitamin E, vitamin C, or β-carotene. Some studies indicated that an inverse association of Se with prostate cancer is found in subjects with high vitamin E intake (38) or plasma concentration (33), whereas

others found no effect modification by vitamin E (32, 37, 39). We also found no effect modification by tertile of intake of vitamin E, vitamin C, or β-carotene from food. Additionally, there was no interaction between smoking status and Se status on prostate cancer risk, which is supported by some (37, 39) but not all studies (30, 32, 38).

Potentially, the association between Se status and prostate cancer risk may also be modified by genetic variation in selenoproteins. Large individual variation in selenoprotein expression in response to Se supplementation has been reported (26), and some of these effects have been attributed to genetic polymorphisms in selenoproteins (10, 11). The availability of Se determines selenoprotein synthesis, but not all selenoproteins are affected to the same extent in case of suboptimal supply (41, 42). We evaluated effect modifications by SNPs of selected selenoproteins and observed a significant interaction for a polymorphism in *GPX1* (rs1050450). *GPX1* encodes for a ubiquitous cellular enzyme that detoxifies hydrogen peroxide; the C>T change leads to a proline to leucine substitution in the protein. Erythrocyte GPx activity has been found to be significantly higher for those with the more common genotype (CC; ref. 15), and the correlation of plasma Se concentration with erythrocyte GPx1 activity was stronger in individuals of CC subjects than in TT subjects (43). The influence of *GPX1* (rs1050450) genotype on erythrocyte GPx1 activity was not confirmed in two small studies (17, 44), but an experimental study with breast cancer cells transfected with either the proline-containing or leucine-containing allele showed a greater induction of enzyme activity by Se in cells homozygous for the common allele (CC; ref. 45). Thus, it seems that carriers of the leucine variants in *GPX1* are hampered in terms of responsiveness to Se. Therefore, higher Se status might be more beneficial for these subjects. This interpretation is in line with our finding that carriers of the leucine (T) allele had a significantly reduced prostate cancer risk with increasing serum Se concentration.

Carriers of the A (GA and AA) in rs7579 in the *SEPP1* gene had higher serum concentrations of SePP compared with homozygous GG individuals. The few data in the literature do not allow for direct comparison of results (10).

In addition, we found an association of borderline statistical significance ($P = 0.05$) between genotype for rs7579 in *SEPP1* and prostate cancer risk. SePP has a well-defined function in transport of Se from the liver to other tissue (46) but is also synthesized in the local target tissues, and rs7579 has been shown to influence the proportion of SePP isoforms in plasma (47). We propose that rs7579 alters the ability to supply Se to the prostate and so influences prostate cancer risk, although we cannot exclude the possibility that the SNP affects SePP synthesis within the prostate.

Familial clustering of prostate cancer (48) indicates that genetic variations play a role in the development of the

Table 5. OR and 95% CI for association of serum selenium concentration (per 10 μg/L) and prostate cancer in strata of genetic polymorphisms in the EPIC-Heidelberg nested case-control study (*n* = 740)

		Total prostate cancer		High-grade prostate cancer	
		Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)
SEP15 G1125A (rs5859)	GG	162/307	0.91 (0.81, 1.02)	63/113	0.86 (0.72, 1.03)
	GA + AA	82/183	0.96 (0.80, 1.14)	27/67	0.95 (0.70, 1.30)
$P_{interaction}$			0.27		0.32
SEP15 C>T (rs540049)	CC	162/323	0.90 (0.80, 1.01)	63/117	0.85 (0.71, 1.02)
	CT + TT	82/167	0.98 (0.82, 1.16)	27/63	0.99 (0.73, 1.35)
$P_{interaction}$			0.18		0.18
SEPP1 G>A coding (rs3877899)	GG	150/270	0.92 (0.81, 1.05)	50/106	0.87 (0.69, 1.10)
	GA + AA	94/220	0.93 (0.79, 1.08)	40/74	0.91 (0.74, 1.13)
$P_{interaction}$			0.82		0.90
SEPP1 G>A 3′UTR (rs7579)	GG	115/250	0.92 (0.80, 1.07)	41/88	0.89 (0.70, 1.13)
	GA + AA	129/240	0.92 (0.80, 1.06)	49/92	0.88 (0.70, 1.11)
$P_{interaction}$			0.90		0.82
GPX1 C>T (rs1050450)	CC	120/263	1.03 (0.89, 1.18)	52/92	1.11 (0.90, 1.37)
	CT+TT	123/222	0.87 (0.76, 0.99)	38/85	0.64 (0.49, 0.83)
$P_{interaction}$			0.03		<0.001
GPX4 C718T (rs713041)	CC	76/155	0.88 (0.73, 1.06)	33/51	0.82 (0.63. 1.07)
	CT + TT	166/333	0.95 (0.84, 1.06)	57/127	0.96 (0.79, 1.17)
$P_{interaction}$			0.76		0.17

NOTE: $P_{interaction}$, *P* value of test for interaction between serum selenium concentration and genotype.

*Unconditional logistic regression adjusted for matching factors (age and time of blood collection) and family history of prostate cancer, participation in PSA testing, smoking status, and vigorous physical activity.

disease, but common polymorphisms (such as the ones studied here) are likely to have a small effect on overall prostate cancer risk when SNPs were considered individually. Furthermore, due to the very limited power of our study, our findings can only be interpreted as first hint that needs confirmation in studies with sufficient statistical power. Additionally, results from genome-wide association studies do not support our finding of an association of rs7579 in SEPP1 and prostate cancer (49).

For the two SNPs in the *SEP15* gene, serum GPx3 activity was lower in carriers of the homozygous less frequent allele; however, this group comprised only 25 subjects, thus warranting a careful interpretation of this finding. In agreement with our data, also recent results from the Physicians' Health Study showed that SEP15 SNPs were not significantly associated with serum selenium levels or with prostate cancer risk (no measurement of GPx3 activity); however, they got indication for effect modification of the association between serum Se and prostate cancer mortality by SEP15 gene variants (variants not included in our study; ref. 50).

In this nested case-control study, we could make use of biospecimens collected before prostate cancer diagnosis. The high response rates achieved during the three completed follow-up rounds (>90%) of the EPIC-Heidelberg cohort minimize the risk of selection bias. However, a limitation of our study is the relatively short follow-up time; thus, we cannot rule out that subclinical prostate cancer at baseline might have affected Se status, an influence which was indicated recently in a case-control study of German men (51). Therefore, we repeated analysis after exclusion of all cases (and matched controls) diagnosed within the first year of follow-up, but still found similar results. We decided on measuring several markers of Se status and showed a good correlation between them. However, Se status was only measured at a single point in time, which was assumed to be representative of the preceding years. An additional strength of our study is that we were able to account for several known or suspected confounding factors and affect modifiers assessed at baseline like smoking status or PSA testing. However, residual confounding cannot be completely ruled out. The size of our study population was determined by the number of incident prostate cancer cases occurred by April 2007. To improve power we matched two controls per cases and did analyses stratified by case set. Although we were able to determine some diet-gene interaction effects, we might not have had enough power to detect some associations with smaller effects. A further limitation is that we had no blinded quality control samples for the analysis of serum Se concentration, Gpx3 activity, or SePP concentration; for the latter two, even certified standard solutions with known content are not available.

In summary, this study showed a borderline significant association between rs7579 in *SEPP1* and prostate cancer risk, and a borderline significant association between serum Se concentration and prostate cancer risk, which was modulated by rs1050450 in the *GPX1* gene, suggesting a role of SePP and GPx1 in prostate function. Future studies should address functional effects of these polymorphisms (e.g., measurements of GPx1 activity) in the prostate so as to further explore the underlying biological mechanisms. Additionally, larger studies are needed to investigate the complex interplay of polymorphisms in different selenoproteins.

Table A1. Association between serum selenoprotein P concentration and prostate cancer risk in the EPIC-Heidelberg nested case-control study (n = 740)

	Quartiles of serum SepP concentration (mg/L)				Continuous (per 1 mg/L)
	<2.58	2.58-2.90	2.90-3.24	≥3.24	
Cases/controls (*n*)	61/121	53/124	74/119	59/127	
Crude OR* (95% CI)	1.00	0.84 (0.54, 1.33)	1.22 (0.79, 1.88)	0.91 (0.57, 1.44)	0.85 (0.62, 1.17)
Adjusted OR† (95% CI)	1.00	0.83 (0.52, 1.33)	1.07 (0.68, 1.68)	0.83 (0.51, 1.33)	0.77 (0.55, 1.07)
Excluding cases diagnosed during 1st year of follow-up (and matched controls)					
Cases/controls (*n*)	58/116	50/116	68/115	58/118	
Adjusted OR† (95% CI)	1.00	0.85 (0.53, 1.36)	1.00 (0.63, 1.59)	0.88 (0.54, 1.43)	0.79 (0.56, 1.11)
High-grade prostate cancer					
Cases/controls (*n*)	25/49	18/34	28/41	20/58	
Adjusted OR† (95% CI)	1.00	0.98 (0.46, 2.06)	1.13 (0.55, 2.29)	0.57 (0.27, 1.22)	0.62 (0.37, 1.05)
Low-grade prostate cancer					
Cases/controls (*n*)	33/66	27/71	37/64	33/56	
Adjusted OR† (95% CI)	1.00	0.75 (0.39, 1.44)	1.08 (0.57, 2.04)	1.07 (0.55, 2.11)	0.89 (0.56, 1.42)

*Crude OR from conditional logistic regression stratified by matching factors (age group and time of recruitment).

†Adjusted OR additionally adjusted for family history of prostate cancer, participation in PSA testing, smoking status, and vigorous physical activity.

Table A2. Association between serum GPx3 activity and prostate cancer risk in the EPIC-Heidelberg nested case-control study (n= 740)

	Quartiles of serum GPx3 activity (units/L)				Continuous (per 100 units/L)
	<589	589-655	655-715	≥715	
Cases/controls (n)	70/121	65/125	48/123	65/123	0.29
		0.57	0.07	0.68	0.93
Crude OR* (95% CI)	1.00	0.89 (0.58, 1.35)	0.67 (0.43, 1.04)	0.92 (0.60, 1.40)	(0.81, 1.07)
		0.36	0.05	0.46	0.17
Adjusted OR† (95% CI)	1.00	0.82 (0.53, 1.26)	0.63 (0.39, 0.99)	0.85 (0.55, 1.31)	0.91 (0.79, 1.04)
Excluding cases diagnosed during 1st year of follow-up (and matched controls)					
Cases/controls (n)	68/116	63/116	44/119	60/155	
Adjusted OR† (95% CI)	1.00	0.84 (0.54, 1.31)	0.59 (0.36, 0.94)	0.83 (0.53, 1.30)	0.88 (0.76, 1.02)
High-grade prostate cancer					
Cases/controls (n)	27/45	26/48	21/44	18/45	
Adjusted OR† (95% CI)	1.00	0.86 (0.43, 1.73)	0.82 (0.41, 1.65)	0.68 (0.33, 1.40)	0.85 (0.67, 1.09)
Low-grade prostate cancer					
Cases/controls (n)	39/66	32/65	23/66	36/61	
Adjusted OR† (95% CI)	1.00	0.74 (0.40, 1.36)	0.50 (0.25, 0.99)	0.80 (0.42, 1.52)	0.90 (0.74, 1.10)

*Crude OR from conditional logistic regression stratified by matching factors (age group and time of recruitment).
†Adjusted OR additionally adjusted for family history of prostate cancer, participation in PSA testing, smoking status, and vigorous physical activity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

German Federal Ministry of Education and Research grant FK 0313846A and Deutsche Krebshilfe grant 10-1793 Scholl. Basic support of the EPIC-Heidelberg cohort study was provided by German Cancer Aid and "Europe Against Cancer" Programme (European Commission, DG SANCO). C. Méplan was supported by NuGO, the EU-funded network in Nutrigenomics. The following authors have membership on EU NoE, which was instrumental in the initiation of the study: J. Linseisen, S. Rohrmann (ECNIS), J. Hesketh (NuGO), and B. Åkesson (NuGO, ECNIS).

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Received 04/06/2010; revised 08/09/2010; accepted 08/30/2010; published OnlineFirst 09/17/2010.

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