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Variation in selenoenzyme genes and prostate cancer risk and survival

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Abstract

Background—While several studies showed that selenium may prevent prostate cancer (PCa), few studies have evaluated variation in selenoenzyme genes in relation to PCa risk and survival.

Methods—We studied common variants in seven selenoenzymes genes in relation to risk of PCa and PCa-specific mortality (PCSM). In a population-based case-control study of men of European ancestry (1,309 cases, 1,266 controls), we evaluated 35 common, tagging single nucleotide polymorphisms (SNPs) in *GPX1* (n = 2), *GPX2* (n = 4), *GPX3* (n = 6), *GPX4* (n = 6), *SEP15* (n = 4), *SEPP1* (n = 6), and *TXNRD1* (n = 7) in relation to PCa risk, and among cases, associations between these variants and risk of PCSM. We used logistic regression and Cox proportional hazards regression to estimate the relative risk of PCa and PCSM, respectively.

Results—Of the SNPs examined, only *GPX1* rs3448 was associated with overall PCa risk with an odds ratio of 0.62 for TT versus CC (95% confidence interval, 0.44–0.88). SNPs in *GPX2*, *GPX3*, *GPX4*, *SEP15*, and *SEPP1* had different risk estimates for PCa in subgroups based on stage and grade. We observed associations between SNPs in *GPX4* and *TXNRD1* and risk of PCSM. None of these associations, however, remained significant after adjustment for multiple comparisons.

Conclusions—We found evidence that genetic variation in a subset of selenoenzyme genes may alter risk of PCa and PCSM. These results need validation in additional subsets.

Keywords

prostate cancer; risk; mortality; selenoenzyme genes; genetic variation

Introduction

Selenium is an essential trace element that may prevent prostate carcinogenesis. Initial evidence for this relationship comes from the Nutritional Prevention of Cancer (NPC) trial,

which in a secondary analysis showed that men randomized to selenium supplementation had a lower risk of prostate cancer (PCa) (1,2). This result prompted the initiation of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which included 35,533 men and, showed no association of selenium supplementation with a reduced PCa risk (3). The SELECT study was stopped early because of the high likelihood that further supplementation would not change interim findings. There have been a number of observational studies of PCa and blood or toenail selenium status and several of these showed associations with a reduced PCa risk (4,5). Evidence is particularly strong for advanced/aggressive PCa, suggesting that selenium might be effective in delaying onset of these clinically relevant cancers (4).

Selenium is incorporated as selenocysteine at the active site of a wide range of proteins and exerts important biological functions through its presence in these selenoproteins (6,7). Most selenoproteins exhibit enzymatic redox function via selenocysteine, which confers their catalytic or antioxidant activities. The human selenoproteome consists of 17 selenoprotein families (6). One major family comprises the glutathione peroxidases, which are antioxidant enzymes that can directly reduce hydrogen peroxide and other reactive oxygen species that potentially contribute to the development and progression of several cancers (8,9). Several other selenoproteins may have anticancer activity (7).

Despite several lines of evidence suggesting that selenium may prevent PCa, variation in selenoenzyme genes in relation to PCa risk has only been studied to a limited extent. A few genetic studies have investigated associations between specific single nucleotide polymorphisms (SNPs) in selenoenzyme genes and risk of PCa, with statistical associations reported for SNPs in glutathione peroxidase 1 (*GPX1*), (10) and selenoprotein P (*SEPP1*) (11). A more recent prospective study showed that SNPs in *SEP15* (15-kDa selenoprotein) were associated with risk of prostate cancer-specific mortality (PCSM) (12).

To further address this issue we conducted a candidate gene study to test the hypothesis that common genetic variation in selenoenzyme genes is related to risk of PCa and PCSM. The genes selected for this analysis are glutathione peroxidase 1–4 (*GPX1*, *GPX2*, *GPX3*, and *GPX4*), *SEP15*, selenoprotein P (*SEPP1*), and thioredoxin reductase 1 (*TXNRD1*). We selected tagging SNPs to capture the underlying genetic variation across each of these genes, and examined associations for the tagging SNPs with PCa and PCSM.

Materials and Methods

Study population

Study participants were men of European ancestry enrolled in one of two population-based PCa case-control studies (13,14). Cases ($n = 1,548$) were diagnosed with histologically confirmed adenocarcinoma of the prostate during two study periods, either January 1, 1993 to December 31, 1996 (Study I, age range 40–64 years) or January 1, 2002 to December 31, 2005 (Study II, age range 35–74 years). These PCa patients were identified via the Seattle-Puget Sound Surveillance, Epidemiology, and End Results (SEER) Program cancer registry. This registry provided information on Gleason score, tumor stage, serum prostate-specific antigen (PSA) level at diagnosis, and primary therapy for PCa. Controls ($n = 1,529$) were recruited evenly throughout case ascertainment periods using random digit telephone dialing and controls were frequency matched by 5-year age groups. The study was approved by the institutional review board of the Fred Hutchinson Cancer Research Center, and written informed consent was obtained from all participants. Genotyping was approved by the institutional review board of the Intramural Program for the National Human Genome Research Institute.

Variant selection and genotyping

To determine the underlying genetic variation in *GPX1–4*, *SEPP1*, and *TXNRD1*, we used sequencing data from the promoter regions, 5' and 3' untranslated regions, including the selenocysteine insertion sequence (essential for selenocysteine incorporation (15)), as well as all exons and intron/exon boundaries (details are described elsewhere (16,17)). The criteria used to select tagging SNPs (tagSNPs) were haplotype $r^2 \geq 0.9$ and minor allele frequency (MAF) $> 5\%$ in European descent population. The tagSNP selection for *SEPP1* was based on publicly available HapMap consortium data (www.hapmap.org; haplotype $r^2 \geq 0.8$ and MAF $\geq 5\%$ in Caucasians), rather than sequence data. In total, 35 tagSNPs (*GPX1*, $n = 2$; *GPX2*, $n = 4$; *GPX3*, $n = 6$; *GPX4*, $n = 6$; *SEPP1*, $n = 6$; and *TXNRD1*, $n = 7$) were genotyped.

Applied Biosystems (ABI) SNPlex™ Genotyping System was used for genotyping, and proprietary GeneMapper software was used for calling alleles (www.appliedbiosystems.com). Specific SNP alleles were determined by the ABI 3730xl DNA Analyzer, based on presence of a unique sequence assigned to each original allele-specific oligonucleotide. Quality control included genotyping of blind duplicate samples (about 11% of samples per SNP), which revealed $>99\%$ agreement on genotyping calls across all SNPs assayed. Each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to case-control and vital status of samples. Genotype frequencies among controls were consistent with Hardy-Weinberg proportions ($P > 0.05$), except for *GPX3* rs8177449 and *SEPP1* rs12055266 (both $P < 0.01$), which were excluded from further analysis.

Prostate cancer-specific survival

Prostate cancer-specific deaths were ascertained from the SEER registry, which links quarterly with the Washington State mortality database and annually with the National Death Index. The completeness of survival follow-up through linkage with these registries was estimated to be nearly 100%. Underlying cause of death obtained from the registries was verified by a review of death certificates, with over 99% agreement. The date of last follow-up for survival was November 1, 2011.

Statistical analyses

For each SNP we classified homozygote carriers of the common allele as the referent group and heterozygote and homozygote carriers of the less common variant allele as the two exposure groups as an unordered categorical variable (which we term the 'co-dominant model'). Trend tests ('trend models'), which used a single indicator variable coded as the number of variant alleles for each SNP, were used to assess allele dosage. Unconditional logistic regression was used to calculate odds ratios (ORs) for the associations between SNP genotypes and PCa risk. Cox proportional hazards regression was used to obtain hazard ratios (HRs) for the associations between SNP genotypes and risk of PCSM. The proportional hazards assumption was tested using the scaled Schoenfeld residuals (18). The variables in tables 1 (logistic regression) and 5 (Cox regression) were selected as potential confounders. Each variable was tested to potentially be included in the statistical models if it changed the beta coefficient (compared to the age-adjusted model) by at least 10%. Because none did so, we report age-adjusted models only.

Multiple comparisons were accounted for by using permutations to calculate exact P-values for each SNP. For each permutation, co-dominant and trend models were fit for all SNPs and the minimum P-values kept for each SNP. P-values were ordered to approximate the null distribution of the order statistics, that is, minimum P-value, second smallest P-value, etc. The original P-values were also ordered and permutation P-values were calculated by

comparing the ordered P-values to the null distribution for the appropriate order statistic. Permutation P-values can be interpreted as the probability of observing a P-value less than or equal to what was observed for the given order statistic under the null hypothesis of no association with risk for any of the 35 SNPs. In permutations, the SNP vector was randomly permuted between subjects so all SNPs for a given subject were kept together, which maintained the LD structure between SNPs. A SNP was considered to be significantly associated with PCa risk if both nominal and permuted P-values were less than 0.05. All P-values were two-sided. Plink (version 1.07) was used to generate linkage disequilibrium (LD) estimates. Analyses were done using the STATA statistical package (version 10.1, STATA Corp., College Station, TX) and R (version 2.10).

Results

The final study population included 1,309 PCa cases and 1,266 control subjects. Cases compared to controls were more likely to have a first-degree family history of PCa and to have had PSA screening prior to reference date (both $P < 0.01$) (Table 1).

When considering all of the tagSNPs, only *GPX1* rs3448 was associated with overall PCa risk with an OR of 0.62 for TT versus CC (95% confidence interval (CI), 0.44–0.88; P for trend = 0.07) (Table 2). This association was significant for both low grade (Gleason 2–7(3+4)) and high grade (Gleason 7(4+3)–10) PCa, with ORs for TT versus CC of 0.66 (95% CI, 0.46–0.94; P for trend = 0.17) and 0.44 (95% CI, 0.20–0.98; P for trend = 0.06), respectively (Table 3). Furthermore, the association with *GPX2* rs4902346 was stronger for high grade PCa compared to low grade PCa, with an OR of high grade PCa of 2.35 for GG versus AA (95% CI, 1.29–4.29; P for trend = 0.08). This SNP was in almost complete LD ($r^2 = 0.98$) with another tagSNP (*GPX2* rs12172810) and, for that reason, we excluded the rs12172810 SNP from further analysis.

The association of *GPX1* rs3448 with PCa was nominally significant for both local stage and regional/distant stage PCa, with ORs for TT versus CC of 0.67 (95% CI, 0.46–0.96; P for trend = 0.12) and 0.47 (95% CI, 0.24–0.93; P for trend = 0.20), respectively (Table 4). One SNP in *GPX3* (rs8177447) was shown to be associated with regional/distant stage but not local stage PCa with an OR of 1.67 for TT versus CC (0.82–3.38; P for trend = 0.04). Two SNPs in *SEPP1* (rs3797310 and rs3877899) were associated with regional/distant stage but not local stage PCa with an OR of 1.68 for TT versus CC (95% CI, 1.10–2.59; P for trend = 0.10) and an OR of 0.33 for AA versus GG (95% CI, 0.13–0.84; P for trend = 0.30), respectively. *GPX4* rs2075710 and *SEP15* rs561104 were associated with local stage but not regional/distant stage PCa with an OR of 1.45 for TT versus CC (95% CI, 1.01–2.10; P for trend = 0.28) and an OR of 1.28 for GG versus AA (95% CI, 0.99–1.64, P for trend = 0.03), respectively. None of these tagSNPs retained significance after adjustment for multiple comparisons.

Among PCa cases, 81 men died of PCa (6.2%) during a median follow-up of 9.1 years (interquartile range: 7.2–15.8). Table 5 shows the baseline characteristics of PCa cases by PCa-specific survival. Men that died of PCa were younger at diagnosis ($P = 0.01$), less likely to have a first-degree family history of PCa ($P = 0.02$), and less likely to have had PSA screening ($P < 0.01$). As expected, fatal PCa was associated with higher Gleason score, advanced stage, and higher PSA value at diagnosis (all $P < 0.01$). With regard to primary treatment, patients that died of PCa were less likely to have had radical prostatectomy and more likely to have had androgen deprivation therapy ($P < 0.01$).

GPX4 rs2074452 was associated with PCSM with an OR of 3.19 for TT versus CC (95% CI, 1.03–9.93, P for trend = 0.12). Three SNPs in *TXNRD1* (rs10778322, rs1128446,

rs4964785) were associated with a lower risk of PCSM for the heterozygous genotype with ORs of 0.57 (95% CI, 0.35–0.96, P for trend = 0.27), 0.58 (95% CI, 0.34–1.00, P for trend = 0.20), and 0.55 (95% CI, 0.33–0.91, P for trend = 0.42), respectively. None of these tagSNPs retained significance after adjustment for multiple comparisons.

Discussion

In this study of genetic variation in selenoenzyme genes in relation to PCa risk and disease-specific survival, we observed an association between a SNP in *GPXI* and risk of PCa. Polymorphisms in *GPX2*, *GPX3*, *GPX4*, *SEP15*, and *SEPP1* had risk estimates that differed for subgroups based on stage and grade. We observed associations between SNPs in *GPX4* and *TXNRD1* and risk of PCSM. None of these associations, however, remained significant after adjustment for multiple comparisons.

Glutathione peroxidases are a family of antioxidant enzymes that remove reactive oxygen species (8). In our study, *GPXI* rs3448 was associated with overall PCa risk and we found some evidence that this association was more pronounced for high grade and advanced stage PCa. *GPXI* rs3448 is located in the 5' region and the biological consequence of this polymorphism is unknown. There is some evidence from prior studies that a functional *GPXI* candidate variant (rs1050450) is involved in PCa (10,11). This polymorphism is not correlated with *GPXI* rs3448 ($r^2 = 0.02$). *GPXI* rs1050450 resides in the coding region and results in an amino acid substitution of proline with leucine (Pro200Leu), (19) and a number of studies showed that this polymorphism is associated with altered GPX activity (20).

To date, no observational study of PCa has specifically evaluated associations for common variation in *GPX2–4*. We observed an association between *GPX2* rs4902346, located in the only intron of this gene, and risk of high grade PCa (Gleason 7(4+3)–10). Interestingly, one study reported a positive correlation between tissue GPX activity and Gleason score (21). This result, which is not in line with the hypothesis that antioxidant enzymes reduce PCa risk, is supported by some studies showing that antioxidants contribute to tumor progression and can cause malignant cells to evade apoptosis (21). Although we had no data on GPX activity, our results suggest that genetic variation in *GPX2* may be associated with tumor aggressiveness. Since we had limited power for the subgroup analysis by tumor grade, this remains to be confirmed in future large-scale studies.

We additionally observed an association between *GPX3* rs8177447, located in intron 4, and risk of advanced stage PCa. This finding is supported by a laboratory study that showed that *GPX3* is often downregulated in PCa samples and that inactivation of *GPX3* correlates with poor clinical outcomes (22). That study additionally showed that overexpression of *GPX3* in PCa cell lines induced the suppression of colony formation and anchorage-independent growth (22).

Selenoprotein P (SEPP1) contains up to 10 selenocysteines and functions as selenium transporter to the target tissue, intracellular selenium binding and supply, and protection against oxidative stress (23,24). Studies using cell lines have shown that the expression of *SEPP1* is often downregulated in PCa, (25) and that this affects oxidative stress levels (26). It has also been shown that PCa is associated with reduced serum selenium P concentrations (27). In our study, *SEPP1* rs3877899 and rs3797310 were associated with high grade PCa. *SEPP1* rs3877899 is present within the protein coding region of the gene and it is predicted to cause an alanine to threonine change (Ala234Thr). *SEPP1* rs3797310 is located in the 5' region. Two prior studies, conducted among European men with a relatively low selenium status, have investigated associations between *SEPP1* rs3877899 and PCa risk and found no associations with either risk of overall PCa (11,28) or features of more aggressive disease

(28). Steinbrecher et al. previously showed a borderline significant association between *SEPP1* rs7579 (AA versus GG) and PCa risk (OR = 1.72; 95% CI, 0.99–2.98) (11). *SEPP1* rs7579, which is located in the 3′ untranslated region, was also one of the tagSNPs evaluated in our study, and, interestingly, we observed a non-significant association with regional/distant stage PCa (OR for AA versus GG = 1.53; 95% CI, 0.99–2.36; P for trend = 0.21). Laboratory studies conducted by others have shown that both *SEPP1* rs3877899 and rs7579 influence the proportion of SEPP isoforms in plasma (29). Our results provide further evidence that *SEPP1* may affect prostate carcinogenesis. These findings need further confirmation.

Although the exact function of the selenoprotein SEP15 is not known, several potentially important biological activities have been suggested (12). *SEP15* has been found to be highly expressed in the prostate (30). Penney et al. recently investigated associations between tagSNPs in *SEP15* and risk of PCa and PCSM (12). Similar to our study, Penney et al. observed no association with risk of overall or more aggressive PCa, but unique from our study, they observed that several tagSNPs were associated with risk of PCSM, suggesting that SEP15 may be involved in tumor progression. Both our study and Penney et al. had an extended follow-up period for assessing PCSM (median follow-up of 9.1 and 10 years, respectively), but our study had fewer events (81 compared to 178 events), which could explain the different findings.

Observational studies of PCa-specific survival have not evaluated common variation in other selenoenzyme genes. We showed that *GPX4* rs2074452, which is located in the 3′ region, was modestly associated with risk of PCSM. Unfortunately, the genotyping of this SNP failed in one of our two case-control studies (Study I), which limited power for this specific analysis. We additionally observed that three polymorphisms in *TXNRD1* (rs10778322, rs1128446, rs4964785) were modestly associated with PCSM. These SNPs were in moderate LD ($r^2 = 0.5$ – 0.6), suggesting that these SNPs may point to a common underlying functional variant, if findings are replicated.

Although we combined two study populations to obtain a large number of cases and controls, we had limited power for the subgroup analyses based on stage and grade and the PCa-specific survival analysis. We used resequencing data for the tagSNP selection (all genes except *SEP15*) to allow a more comprehensive analysis of the common genetic variation. We did not, however, have the resources to resequence entire genes, making it possible that some of the genetic variation was missed. Nonetheless, our resequencing effort was focused on the most functionally relevant regions of the genes (promoter, coding regions, intron/exon boundaries, and selenocysteine insertion sequence). We did not have data on serum or nail selenium concentration, and selenium-SNP interactions were therefore not evaluated. Most men in our US-based study population are assumed to have an adequate selenium status with only a few men expected to have a low status (7). It is believed that certain variants in selenoenzyme genes only affect the enzyme function among individuals with selenium deficiency and therefore, even if data on selenium status were available in our study, we would have too few men of low selenium status and insufficient power to investigate SNP-associations in this subgroup. Future studies of genetic variation in selenoenzyme genes and PCa should consider focusing on men with a low selenium status.

In summary, we found evidence that genetic variation in a subset of selenoenzyme genes may be associated with overall risk of PCa, PCa risk in subgroups based on stage and grade, and PCSM. As this is the first study of PCa to comprehensively analyze selenium pathway gene variants, further studies are needed to replicate these findings.

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Abbreviations

PCa	prostate cancer
PCSM	prostate cancer-specific mortality
PSA	prostate-specific antigen
SNP	single nucleotide polymorphism

References

1. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Leshner JL Jr, Park HK, Sanders BB Jr, Smith CL, Taylor JR. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA. 1996; 276(24): 1957–1963. [PubMed: 8971064]
2. Duffield-Lillico AJ, Dalkin BL, Reid ME, Turnbull BW, Slate EH, Jacobs ET, Marshall JR, Clark LC. Nutritional Prevention of Cancer Study G. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU Int. 2003; 91(7):608–612. [PubMed: 12699469]
3. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD 3rd, Crawford ED, Goodman GE, Claudio J, Winkquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr, Baker LH, Coltman CA Jr. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA. 2009; 301(1):39–51. [PubMed: 19066370]
4. Hurst R, Hooper L, Norat T, Lau R, Aune D, Greenwood DC, Vieira R, Collings R, Harvey LJ, Sterne JA, Beynon R, Savovic J, Fairweather-Tait SJ. Selenium and prostate cancer: systematic review and meta-analysis. American Journal of Clinical Nutrition. 2012; 96(1):111–122. [PubMed: 22648711]
5. Peters U, Takata Y. Selenium and the prevention of prostate and colorectal cancer. Mol Nutr Food Res. 2008; 52(11):1261–1272. [PubMed: 18763256]
6. Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal. 2007; 9(7):775–806. [PubMed: 17508906]
7. Rayman MP. Selenium and human health. Lancet. 2012; 379(9822):1256–1268. [PubMed: 22381456]
8. Brigelius-Flohe R, Kipp A. Glutathione peroxidases in different stages of carcinogenesis. Biochim Biophys Acta. 2009; 1790(11):1555–1568. [PubMed: 19289149]
9. Halliwell B. Oxidative stress and cancer: have we moved forward? Biochem J. 2007; 401(1):1–11. [PubMed: 17150040]
10. Arsova-Sarafinovska Z, Matevska N, Eken A, Petrovski D, Banev S, Dzikova S, Georgiev V, Sikole A, Erdem O, Sayal A, Aydin A, Dimovski AJ. Glutathione peroxidase 1 (GPX1) genetic polymorphism, erythrocyte GPX activity, and prostate cancer risk. Int Urol Nephrol. 2009; 41(1): 63–70. [PubMed: 18563616]
11. Steinbrecher A, Meplan C, Hesketh J, Schomburg L, Endermann T, Jansen E, Akesson B, Rohrmann S, Linseisen J. Effects of selenium status and polymorphisms in selenoprotein genes on prostate cancer risk in a prospective study of European men. Cancer Epidemiol Biomarkers Prev. 2010; 19(11):2958–2968. [PubMed: 20852007]

12. Penney KL, Schumacher FR, Li H, Kraft P, Morris JS, Kurth T, Mucci LA, Hunter DJ, Kantoff PW, Stampfer MJ, Ma J. A large prospective study of SEP15 genetic variation, interaction with plasma selenium levels, and prostate cancer risk and survival. *Cancer Prev Res (Phila)*. 2010; 3(5): 604–610. [PubMed: 20424130]
13. Agalliu I, Salinas CA, Hansten PD, Ostrander EA, Stanford JL. Statin use and risk of prostate cancer: results from a population-based epidemiologic study. *American Journal of Epidemiology*. 2008; 168(3):250–260. [PubMed: 18556686]
14. Stanford JL, Wicklund KG, McKnight B, Daling JR, Brawer MK. Vasectomy and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 1999; 8(10):881–886. [PubMed: 10548316]
15. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zhehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. *Science*. 2003; 300(5624):1439–1443. [PubMed: 12775843]
16. Foster CB, Aswath K, Chanock SJ, McKay HF, Peters U. Polymorphism analysis of six selenoprotein genes: support for a selective sweep at the glutathione peroxidase 1 locus (3p21) in Asian populations. *BMC Genet*. 2006; 7:56. [PubMed: 17156480]
17. Peters U, Chatterjee N, Hayes RB, Schoen RE, Wang Y, Chanock SJ, Foster CB. Variation in the selenoenzyme genes and risk of advanced distal colorectal adenoma. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(5):1144–1154. [PubMed: 18483336]
18. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994; 81:515–526.
19. Moscow JA, Schmidt L, Ingram DT, Gnarr J, Johnson B, Cowan KH. Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. *Carcinogenesis*. 1994; 15(12): 2769–2773. [PubMed: 8001233]
20. Takata Y, King IB, Lampe JW, Burk RF, Hill KE, Santella RM, Kristal AR, Duggan DJ, Vaughan TL, Peters U. Genetic variation in GPX1 is associated with GPX1 activity in a comprehensive analysis of genetic variations in selenoenzyme genes and their activity and oxidative stress in humans. *Journal of Nutrition*. 2012; 142(3):419–426. [PubMed: 22259188]
21. Jerome-Morais A, Wright ME, Liu R, Yang W, Jackson MI, Combs GF Jr, Diamond AM. Inverse association between glutathione peroxidase activity and both selenium-binding protein 1 levels and Gleason score in human prostate tissue. *Prostate*. 2012; 72(9):1006–1012. [PubMed: 22072582]
22. Yu YP, Yu G, Tseng G, Cieply K, Nelson J, Defrances M, Zarnegar R, Michalopoulos G, Luo JH. Glutathione peroxidase 3, deleted or methylated in prostate cancer, suppresses prostate cancer growth and metastasis. *Cancer Res*. 2007; 67(17):8043–8050. [PubMed: 17804715]
23. Burk RF, Hill KE. Selenoprotein P-expression, functions, and roles in mammals. *Biochim Biophys Acta*. 2009; 1790(11):1441–1447. [PubMed: 19345254]
24. Moschos MP. Selenoprotein P. *Cell Mol Life Sci*. 2000; 57(13–14):1836–1845. [PubMed: 11215510]
25. Calvo A, Xiao N, Kang J, Best CJ, Leiva I, Emmert-Buck MR, Jorcyk C, Green JE. Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. *Cancer Res*. 2002; 62(18):5325–5335. [PubMed: 12235003]
26. Gonzalez-Moreno O, Boque N, Redrado M, Milagro F, Campion J, Endermann T, Takahashi K, Saito Y, Catena R, Schomburg L, Calvo A. Selenoprotein-P is down-regulated in prostate cancer, which results in lack of protection against oxidative damage. *Prostate*. 2011; 71(8):824–834. [PubMed: 21456065]
27. Meyer HA, Hollenbach B, Stephan C, Endermann T, Morgenthaler NG, Cammann H, Kohrle J, Jung K, Schomburg L. Reduced serum selenoprotein P concentrations in German prostate cancer patients. *Cancer Epidemiol Biomarkers Prev*. 2009; 18(9):2386–2390. [PubMed: 19690186]
28. Cooper ML, Adami HO, Gronberg H, Wiklund F, Green FR, Rayman MP. Interaction between single nucleotide polymorphisms in selenoprotein P and mitochondrial superoxide dismutase determines prostate cancer risk. *Cancer Res*. 2008; 68(24):10171–10177. [PubMed: 19074884]

29. Meplan C, Nicol F, Burtle BT, Crosley LK, Arthur JR, Mathers JC, Hesketh JE. Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, se intake, and cancer status. *Antioxid Redox Signal*. 2009; 11(11):2631–2640. [PubMed: 19453253]
30. Kumaraswamy E, Malykh A, Korotkov KV, Kozyavkin S, Hu Y, Kwon SY, Moustafa ME, Carlson BA, Berry MJ, Lee BJ, Hatfield DL, Diamond AM, Gladyshev VN. Structure-expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiology. *J Biol Chem*. 2000; 275(45):35540–35547. [PubMed: 10945981]

Table 1

Baseline characteristics of prostate cancer cases and controls

Variables	Cases n = 1,309	Controls n = 1,266	p*
Age (diagnosis) group (y), %			
35–49	7.8	8.5	
50–54	14.4	14.1	
55–59	24.8	27.1	
60–64	30.2	26.4	
65–69	11.7	12.6	
70–74	11.1	11.4	
First-degree family history of prostate cancer, %	21.6	11.2	<0.01
Body mass index (kg/m ²), %			0.34
<25	32.8	30.7	
25.0–29.9	48.7	48.8	
30	18.5	20.5	
Prostate cancer screening history [†] , %			<0.01
None	10.3	13.2	
Digital rectal exam only	17.1	38.2	
PSA testing	72.6	48.7	
Gleason score, %			
2–4	5.1		
5–6	52.1		
7(3+4)	27.3		
7(4+3)	5.8		
8–10	9.7		
Stage of cancer, %			
Local	78.2		
Regional	19.4		
Distant	2.4		

Abbreviation: PSA, prostate-specific antigen.

* A chi-square test was used.

[†] Prostate cancer screening within the 5-year period before the reference date (date of diagnosis for cases and an assigned date for controls that approximated the distribution of the diagnosis dates of cases).

Table 2

Odds ratio (OR) and 95% confidence interval (CI) for the association between *GPX1* rs3448 and risk of overall prostate cancer

Gene	Polymorphism	Genotype	Cases	Controls	OR [*]	95% CI [*]	P trend [†]
<i>GPX1</i>	rs3448	CC	695	665	1.00		
		CT	497	480	0.99	0.84–1.17	
		TT	60	92	0.62	0.44–0.88	0.07

* Adjusted for age (5-year age groups).

† Nominal P-value for linear trend according to the number of (less common) variant alleles.

Odds ratio (OR) and 95% confidence interval (CI) for the association between *GPX1* rs3448 and *GPX2* rs4902346 and risk of prostate cancer stratified by disease grade

Table 3

Gene	Polymorphism	Genotype	Gleason 2-7(3+4)				Gleason 7(4+3)-10			
			Controls	Cases	OR*	95% CI*	P trend†	Cases	OR*	95% CI*
<i>GPX1</i>	rs3448	CC	665	580	1.00			118	1.00	
		CT	480	425	1.01	0.85-1.20		73	0.87	0.64-1.19
		TT	92	53	0.66	0.46-0.94	0.17	7	0.44	0.20-0.98
<i>GPX2</i>	rs4902346‡	AA	810	674	1.00			125	1.00	
		AG	382	337	1.06	0.89-1.27		59	1.01	0.72-1.41
		GG	47	50	1.28	0.85-1.93	0.25	16	2.35	1.29-4.29

* Adjusted for age (5-year age groups).

† Nominal P-value for linear trend according to the number of (less common) variant alleles.

‡ *GPX2* rs4902346 is in almost complete LD ($r^2 = 0.98$) with another tagSNP (*GPX2* rs12172810; not shown).

Table 4

Odds ratio (OR) and 95% confidence interval (CI) for the association between polymorphisms in *GPX1*, *GPX3*, *GPX4*, *SEP15*, and *SEPP1* and risk of prostate cancer stratified by disease stage

Gene	Polymorphism	Genotype	Local stage				Regional/distant stage			
			Controls	Cases	OR*	95% CI*	P trend†	Cases	OR*	95% CI*
<i>GPX1</i>	rs3448	CC	665	545	1.00			150	1.00	
		CT	480	383	0.98	0.82–1.17		114	1.03	0.79–1.36
		TT	92	50	0.67	0.46–0.96	0.12	10	0.47	0.24–0.93
<i>GPX3</i>	rs8177447	CC	866	694	1.00			176	1.00	
		CT	332	250	0.94	0.77–1.14		84	1.27	0.95–1.70
		TT	33	30	1.14	0.69–1.88	0.82	11	1.67	0.82–3.38
<i>GPX4</i>	rs2075710	CC	719	556	1.00			155	1.00	
		CT	471	358	0.98	0.82–1.17		111	1.11	0.85–1.46
		TT	59	67	1.45	1.01–2.10	0.28	10	0.82	0.41–1.65
<i>SEP15</i>	rs561104	AA	439	302	1.00			102	1.00	
		AG	607	499	1.20	0.99–1.45		133	0.94	0.70–1.25
		GG	204	179	1.28	0.99–1.64	0.03	40	0.84	0.56–1.25
<i>SEPP1</i>	rs3797310	CC	589	473	1.00			122	1.00	
		CT	547	401	0.92	0.77–1.09		113	0.98	0.74–1.30
		TT	104	93	1.12	0.82–1.51	0.97	36	1.68	1.10–2.59
<i>SEPP1</i>	rs3877899	GG	739	593	1.00			165	1.00	
		AG	426	338	0.99	0.82–1.18		103	1.08	0.82–1.43
		AA	67	48	0.89	0.60–1.31	0.63	5	0.33	0.13–0.84

* Adjusted for age (5-year age groups).

† Nominal P-value for linear trend according to the number of (less common) variant alleles.

Table 5

Baseline characteristics of prostate cancer patients by prostate cancer-specific survival

Variables	<u>Prostate cancer-specific death</u>		p*
	Yes n = 81	No n = 1,228 [†]	
Mean age at diagnosis, y (SD)	58.0 (8.0)	60.0 (6.9)	0.01
First-degree family history of prostate cancer, %	11.1	22.3	0.02
Body mass index (kg/m ²), %			0.85
<25	34.6	32.7	
25.0–29.9	45.7	48.9	
30	19.8	18.4	
Prostate cancer screening history [‡] , %			<0.01
None	28.4	9.1	
Digital rectal exam only	18.5	17.0	
PSA testing	53.1	73.9	
Gleason score, %			<0.01
2–6	19.0	59.7	
7 (3+4)	25.3	27.4	
7 (4+3)–10	55.7	12.9	
Stage of cancer, %			<0.01
Local	34.6	81.0	
Regional	34.6	18.4	
Distant	30.9	0.6	
PSA value at diagnosis (ng/ml), %			<0.01
<10	30.7	77.5	
10	69.3	22.5	
Primary treatment for prostate cancer, %			<0.01
Radical prostatectomy	28.4	60.8	
Radiation with or without ADT	27.2	27.6	
ADT only	38.3	2.5	
Active surveillance	6.2	8.6	
Other	0.0	0.5	

Abbreviations: SD, standard deviation; PSA, prostate-specific antigen; ADT, androgen deprivation therapy.

* A chi-square test (categorical variables) or t test (continuous variables) was used.

[†] Includes 88 men who died of another cause and were censored at time of death.[‡] Prostate cancer screening within the 5-year period before prostate cancer diagnosis.

Hazard ratio (HR) and 95% confidence interval (CI) for the association between polymorphisms in *GPX4* and *TXNRD1* and risk of prostate cancer-specific mortality

Table 6

Gene	Polymorphism	Genotype	Person-years	No. events	HR*	95% CI*	P trend†
<i>GPX4</i>	rs2074452‡	CC	2,933	12	1.00		
		CT	2,071	10	1.19	0.51–2.75	
		TT	314	4	3.19	1.03–9.93	0.12
<i>TXNRD1</i>	rs10778322	CC	6,783	45	1.00		
		CT	5,750	22	0.57	0.35–0.96	
		TT	1,151	8	1.05	0.50–2.24	0.27
<i>TXNRD1</i>	rs1128446	CC	8,350	53	1.00		
		CG	4,652	17	0.58	0.34–1.00	
		GG	555	4	1.15	0.42–3.17	0.20
<i>TXNRD1</i>	rs4964785	CC	4,280	32	1.00		
		CG	6,932	29	0.55	0.33–0.91	
		GG	2,323	16	0.93	0.51–1.70	0.42

* Adjusted for age (continuous).
† Nominal P-value for linear trend according to the number of (less common) variant alleles.
‡ This SNP failed genotyping in one of the two combined case-control studies (Study I).