Interaction between Single Nucleotide Polymorphisms in Selenoprotein P and Mitochondrial Superoxide Dismutase Determines Prostate Cancer Risk

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Abstract
Selenium may affect prostate cancer risk via its plasma carrier selenoprotein P which shows dramatically reduced expression in prostate cancer tumors and cell lines. The selenoprotein P (SEPP1) Ala234 single nucleotide polymorphism (SNP) allele is associated with lower plasma selenoprotein P in men, reducing the concentration/activity of other antioxidant selenoproteins. Selenium status also modifies the effect of the mitochondrial superoxide dismutase (SOD2) SNP Ala16Val on prostate cancer risk. We investigated the relationship of these SNPs with prostate cancer risk. DNA from 2,975 cases and 1,896 age-matched controls from the population-based Prostate Cancer in Sweden study were genotyped using TaqMan assays. Cases were designated aggressive or nonaggressive prostate cancers at diagnosis by clinical criteria. Association with prostate cancer was investigated by logistic regression; gene-gene interaction using a general linear model. The mean plasma selenium concentration measured in 169 controls was relatively low (76.0 ± 17.2 μg/L). SNP genotype distributions were in Hardy-Weinberg equilibrium. SOD2-Ala16+ men were at a greater risk of prostate cancer [odds ratios (OR), 1.19; 95% confidence intervals (CI), 1.03–1.37] compared with SOD2-Val16 homozygotes. Men homozygous for SEPP1-Ala234 who were also SOD2-Ala16+ had a higher risk of prostate cancer (OR, 1.43; 95% CI, 1.17–1.76) and aggressive prostate cancer (OR, 1.60; 95% CI, 1.22–2.09) than those who were SOD2-Val16 homozygotes (interaction, prostate cancer P = 0.05; aggressive prostate cancer P = 0.01). This interaction was stronger in ever-smokers: SOD2-Ala16+ men homozygous for SEPP1-Ala234 had an almost doubled risk of prostate cancer (OR, 1.97; 95% CI, 1.33–2.91; interaction P = 0.001). In a low-selenium population, SOD2-Ala16+ men homozygous for SEPP1-Ala234 are at an increased risk of prostate cancer/aggressive prostate cancer especially if ever-smokers, because they are likely to produce more mitochondrial H2O2 that they cannot remove, thereby promoting prostate tumor cell proliferation and migration.

Introduction
Selenium, an essential nutrient, may reduce the incidence and/or progression of prostate cancer, particularly in men with the relatively low baseline selenium status commonly found in Europe (1–4). The potential anticaner effects of selenium may be exerted through a number of parallel mechanisms, some of which involve the selenoproteins (1). Indeed, recent evidence suggests an important role for selenoproteins in cancer (1, 5), specifically in prostate cancer (6).

Selenoprotein P contains at least 40% of the total selenium in plasma (7). Deletion of the gene for selenoprotein P in mouse models alters the distribution of selenium in body tissues, suggesting that selenoprotein P is required for selenium transport (8, 9). Although the human selenoprotein P gene (SEPP1) is abundantly expressed in normal colon mucosa, there is a significant reduction or loss of SEPP1 mRNA expression in colon cancers (10). Expression of SEPP1 is also dramatically reduced in a subset of human prostate tumors, mouse tumors, and in the androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cell lines (11). Homozygosity for the Ala234 allele of the SEPP1-Ala234Thr single nucleotide polymorphism (SNP; rs3877899) is associated with a lower concentration of plasma selenoprotein P in men, affecting the concentration and/or activity of other selenoproteins, notably of thioredoxin reductase 1 and some of the antioxidant glutathione peroxidases (GPx; ref. 12). Thus, it is conceivable that the SEPP1 genotype may affect prostate cancer risk.

Selenium status is linked to the effect of another polymorphism on prostate cancer risk, i.e., that of mitochondrial superoxide dismutase (SOD2 or MnSOD; ref. 13), the major detoxifying enzyme in the mitochondrion. This enzyme dismutes superoxide to H2O2, which must itself then be detoxified to water by GPx (13). A well-characterized polymorphism in SOD2 results in the substitution of alanine (Ala) for valine (Val) at codon 16 (rs4880), and a higher activity of the Ala16 mitochondrial enzyme (14). Among men in the U.S. that were homozygous for Ala16, those whose plasma selenium was in the top quartile had a relative risk of prostate cancer of 0.3 (95% CI, 0.2–0.7) and of clinically aggressive prostate cancer of 0.2 (95% CI, 0.1–0.5) when compared with those whose plasma selenium was in the bottom quartile (13). The dependence on selenium status of this genotype effect on prostate cancer risk may relate to the requirement for adequate GPx to remove the extra H2O2 formed in Ala16 homozygotes (13).

We investigated the relationship of these two putatively functional polymorphisms to prostate cancer risk in the large CPS (Prostate Cancer in Sweden) study (15). We hypothesized that in a low-selenium environment, as in Sweden, men who have...
SNP alleles that both reduce their ability to make functional selenoprotein P (SEPP1-Ala234 homozygotes) and increase their production of H2O2 (SOD2-Ala16) thereby increasing their requirement for GPx, would have a greater risk of prostate cancer than men who do not have these alleles.

**Subjects and Methods**

**Participants.** The CAPS study is a large-scale, population-based, prostate cancer case-control study which has been extensively described in previous work (15, 16). The inclusion criterion for cases was a newly diagnosed, pathologically or cytologically verified adenocarcinoma of the prostate. In total, 3,648 prostate cancer patients were invited to participate in the study and 3,161 (87%) agreed. DNA samples were obtained from a total of 2,915 cases, for whom the corresponding clinical data and completed demographic questionnaires were available. Cases were classified as either nonaggressive prostate cancer, nonaggressive prostate cancer, and aggressive prostate cancer in the CAPS study.

**Plasma selenium measurement.** EDTA plasma samples from 169 controls were stored at −80°C prior to determination of selenium by dynamic reaction cell (DRC) inductively coupled plasma mass spectrometry using an Elan 6100 DRC plus (SCIEX Perkin-Elmer). *78Selenium was measured, using methane (at 0.5 mL/min) as the DRC gas to remove the argon dimer and butanol to increase the sensitivity of the signal (18).* Within the plasma selenium concentrations used in this study, the within-run coefficient of variation was 2.1% to 2.6%, whereas the between-run coefficient of variation was 3.1% to 5.6% (n = 10). Accuracy was assured by analysis of four internal quality control serum samples (Trace Elements External Quality Assessment Scheme, University of Surrey, Guildford, Surrey, United Kingdom) and certified reference materials: Seronorm Serum Control Scheme, University of Surrey, Guildford, Surrey, United Kingdom. The University of Surrey research ethics committee approved this genetics study.

**Genotyping.** DNA was extracted from leukocytes using a Puregene kit (Gentra Systems). All genotyping was performed using TaqMan assays and the operator was blinded to case/control status. Controls of known genotype for each of the polymorphisms investigated were included in the assay. Nontemplate controls and duplicate samples were incorporated for quality control purposes. SOD2-Ala16Val (rs4880) PCR primers and dual-labeled, allelic probes were designed and manufactured by Applied Biosystems, Inc. Primers and probes were as follows: forward GCTGGTGCTTCTCTGCTTCCAG, reverse GTGCCCTGAGGCCCAGTAC, Ala16 Probe VIC-CCTGCCAGGCGGC-TAM, Val16 probe FAM-CCAAGGGACGC-TAM.

**Table 1. The relationship between SNPs in two genes, SOD2 (Val16Ala) and SEPP1 (Ala234Thr), with risk of all prostate cancer, nonaggressive prostate cancer, and aggressive prostate cancer in the CAPS study**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>All prostate cancer</th>
<th>Nonaggressive prostate cancer</th>
<th>Aggressive prostate cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>OR (95% CI)*</td>
<td>P</td>
</tr>
<tr>
<td>SOD2 codon 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>1,636</td>
<td>2,634</td>
<td>1.648</td>
<td>Referent</td>
</tr>
<tr>
<td>Ala/Val</td>
<td>789</td>
<td>1,352</td>
<td>0.96 (0.85–1.13)</td>
<td>0.48</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>424</td>
<td>680</td>
<td>1.21 (1.03–1.41)</td>
<td>0.11</td>
</tr>
<tr>
<td>Ala/Val + Ala/Ala</td>
<td>1,213</td>
<td>2,032</td>
<td>1.19 (1.03–1.37)</td>
<td>0.02</td>
</tr>
<tr>
<td>SEPP1 codon 234</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>1,570</td>
<td>2,643</td>
<td>1.653</td>
<td>Referent</td>
</tr>
<tr>
<td>Ala/Thr</td>
<td>878</td>
<td>1,522</td>
<td>0.95 (0.82–1.11)</td>
<td>0.37</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>97</td>
<td>172</td>
<td>1.13 (0.84–1.52)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ala/Thr + Thr/Thr</td>
<td>692</td>
<td>1,121</td>
<td>0.98 (0.85–1.13)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

NOTE: n = number of subjects.
*ORs adjusted for age and geographic location, 95% CI.
assess the association between SNP genotypes and prostate cancer risk, with genotypes coded either as number of rare alleles (0, 1, 2) or as minus allele/plus allele (0, 1) as appropriate, with adjustment for the possible confounding factors age and geographic location.

Genotype-specific risks were estimated as adjusted odds ratios (OR) with associated 95% confidence intervals (CI) by logistic regression. The interaction between the two SNPs in determining prostate cancer risk was assessed using a general linear model.

### Table 2. The interaction between SOD2-Val16Ala and SEPP1-Ala234Thr SNPs and risk of all prostate cancer, nonaggressive prostate cancer, and aggressive prostate cancer in the CAPS study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>All prostate cancer</th>
<th>Nonaggressive prostate cancer</th>
<th>Aggressive prostate cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n OR (95% CI)*</td>
<td>P</td>
<td>n</td>
</tr>
<tr>
<td>SEPP1 codon 234 Ala/Ala</td>
<td>816</td>
<td>1.360</td>
<td></td>
<td>854</td>
</tr>
<tr>
<td>SOD2-Val/Val</td>
<td>224</td>
<td>286</td>
<td>Referent 1</td>
<td>189</td>
</tr>
<tr>
<td>SOD2-Ala/Val + Ala/Ala</td>
<td>592</td>
<td>1.074 1.43 (1.17–1.76) 0.0005</td>
<td>665</td>
<td>1.36 (1.09–1.71) 0.0073</td>
</tr>
<tr>
<td>SEPP1 codon 234 Ala/Thr + Thr/Thr</td>
<td>638</td>
<td>1.009</td>
<td></td>
<td>631</td>
</tr>
<tr>
<td>SOD2-Val/Val</td>
<td>161</td>
<td>246</td>
<td>Referent 1</td>
<td>147</td>
</tr>
<tr>
<td>SOD2-Ala/Val + Ala/Ala</td>
<td>477</td>
<td>763</td>
<td>1.06 (0.84–1.34) 0.62</td>
<td>484</td>
</tr>
<tr>
<td>P for interaction</td>
<td></td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: n = number of subjects.
*ORs adjusted for age and geographic location, 95% CI.

Figure 1. The interaction between SOD2-Val16Ala and SEPP1-Ala234Thr SNPs and risk for all prostate cancers (A), aggressive prostate cancer (B), prostate cancer in never-smokers (C), and prostate cancer in ever-smokers (D) in the CAPS study. ORs adjusted for age and geographic location. R, referent group (foreground column) for each comparison with the alternative SOD2 genotype group (background column).
Weinberg equilibrium and the allele frequencies within the control samples, genotyping error rates were <1.5%. Both the SOD2-Ala16Val and controls, and on tumor classification for the cases, are given in Supplementary Table S1.

**Results**

**Demographics.** Data on age and smoking status for the cases and controls, and on tumor classification for the cases, are given in Supplementary Table S1.

**Selenium status.** The mean (± SD) plasma selenium was 76.0 ± 17.2 μg/L in 169 CAPS control samples, confirming the expected relatively low-selenium status of this group of Swedish men. There was no difference in selenium status between genotypes or by smoking status.

**SNP genotyping.** Through the blinded genotyping of duplicated samples, genotyping error rates were <1.5%. Both the SOD2-Ala16Val and SEPP1-Ala234Thr polymorphisms were shown to be in Hardy-Weinberg equilibrium and the allele frequencies within the control samples confirm the expected relatively low selenium status of this group of Swedish men. There was no difference in selenium status between genotypes or by smoking status.

**SOD2 and SEPP1 genotypes and prostate cancer risk.** Individuals with at least one SOD2-Ala16 allele (SOD2-Ala16+) had an almost 20% increased risk of prostate cancer compared with Val16 homozygotes (adjusted OR, 1.19; 95% CI, 1.03–1.37; \( P = 0.02 \); Table 1). No association between SEPP1-Ala234Thr genotype and prostate cancer risk was observed. The association between the SOD2 polymorphism and either nonaggressive or aggressive disease was of similar magnitude to that with all prostate cancer (nonaggressive plus aggressive; Table 1). There was no association between SEPP1 genotype and either nonaggressive or aggressive disease.

**Interaction between SOD2 and SEPP1 polymorphisms.** Men homozygous for the SEPP1-Ala234 allele, who were also SOD2-Ala16+, were at 43% greater risk of prostate cancer than SOD2-Val16 homozygotes (adjusted OR, 1.43; 95% CI, 1.17–1.76; \( P = 0.0005 \); Table 2; Fig. 1). By contrast, there was no association between SOD2 genotype and cancer risk in SEPP1-Thr234+ men. This interaction between the two SNPs in determining the risk of prostate cancer had a borderline statistically significant value (\( P = 0.05 \)).

In aggressive prostate cancer, the interaction between the SNPs was stronger (\( P = 0.001 \)) with SEPP1-Ala234 homozygotes who were also SOD2-Ala16+ having a 60% increased risk of aggressive disease compared with SOD2-Val16 homozygotes (adjusted OR, 1.60; 95% CI, 1.22–2.09; \( P = 0.0007 \); Table 2; Fig. 1). In never-smokers, the OR associated with SOD2 genotype in the SEPP1-Thr234+ men. Although, the association with SNP genotypes in nonaggressive disease might seem significant, the interaction between the two SNPs was far from statistically significant as shown by the broad overlap between the OR CIs in the two SEPP1 genotype groups (Table 2).

**Effect of smoking on the SOD2 and SEPP1 associations with prostate cancer risk.** Although smoking status was not available for all subjects, given the known effect of smoking on antioxidant status, we investigated the effect of genotype on prostate cancer risk in the subset of participants (CAPS1; ref. 15) for whom smoking data were available (Table 3). Neither SOD2 nor SEPP1 genotype taken separately significantly affected the risk of prostate cancer in either never- or ever-smokers. The OR associated with the SOD2-Ala16+ ever-smokers (Table 3) was, however, similar in magnitude to that in the group as a whole (Table 1) although the association did not reach statistical significance.

The interaction between SEPP1 and SOD2 SNPs in determining prostate cancer risk was modified by smoking status (Table 4; Fig. 1). Ever-smokers homozygous for SEPP1-Ala234 had a highly significant 2-fold increase in prostate cancer risk if they were also SOD2-Ala16+ (OR, 1.97; 95% CI, 1.33–2.91; \( P = 0.0007 \)). The association between SOD2 genotype and cancer risk in ever-smokers was not observed in SEPP1-Thr234+ men (OR, 0.75; 95% CI, 0.47–1.18; \( P = 0.21 \)). This interaction between SEPP1 and SOD2 SNPs in determining prostate cancer risk in ever-smokers was highly significant (\( P = 0.0014 \)), contrasting with the lack of interaction found in never-smokers (\( P = 0.43 \)).

**Discussion**

The mean plasma selenium concentration of 76.0 ± 17.2 μg/L in control samples confirms the relatively low selenium intake and
status in the Swedish population (compared with the mean U.S. value of 125 μg/L determined in the Third National Health and Nutrition Examination Survey; ref. 19). This value is less than the 92 μg/L required for maximal plasma GPx activity (20) and considerably less than the plasma concentration required for full expression of SEPP1 (7, 21), demonstrating that the study population has a selenium intake inadequate for optimal selenoprotein synthesis and/or activity. This may be relevant to prostate cancer risk because low selenoprotein production led to higher-grade lesions and aggressive disease in a transgenic mouse model of prostate cancer (6). We reasoned that in a population with a relatively low selenium status, interindividual variation in selenium requirements, as determined by selenoprotein genotype, might have a greater effect on the risk of prostate cancer than in a selenium-replete population.

Despite our rationale, we found no effect of SEPP1 genotype per se on the risk of prostate cancer or on nonaggressive or aggressive prostate cancer. A similar null effect of this genotype was found in colorectal cancer (10). We did, however, find an effect of genotype in a pathway associated with selenoprotein function: although SOD2 is not a selenoprotein, the product of its activity, H2O2, is a substrate for GPx. The Val16Ala SNP in SOD2 has been shown to alter the secondary structure of the mitochondrial import sequence substrate for GPx. The Val16Ala SNP in SOD2 has been shown to alter the secondary structure of the mitochondrial import sequence of the superoxide dismutase protein such that the Ala16 variant is imported more efficiently into the mitochondrial matrix, resulting in higher enzyme activity (22). Individuals with at least one SOD2-Ala16 allele (Ala16+) therefore generate more active superoxide dismutase (and therefore more H2O2) than those homozygous for the Val16 variant. In our study, SOD2-Ala16+ men were at a 19% increased risk of prostate cancer compared with Val homozygotes (Table 1). The mitochondrion contains little or no catalase and so is entirely dependent on the activity of GPx to remove H2O2 (23), although of course H2O2 is sufficiently long-lived to diffuse out of the mitochondrion.

H2O2 promotes prostate cancer cell proliferation and migration (24, 25), and induces matrix metalloproteinases required for tumor invasion (26, 27). For instance, H2O2 levels rose in cell lines of the LNCaP series as tumorigenic and metastatic potential increased (24). Furthermore, the addition of ebselen, a GPx mimetic, to the assay completely abolished the chemiluminescence attributable to H2O2 (24). At low selenium concentrations, in which there is insufficient GPx activity to remove H2O2, the SOD2-Ala16 allele would therefore be expected to have a deleterious effect owing to the higher H2O2 production associated with that allele variant. Our population has a mean plasma selenium concentration well below the level required to fully optimize GPx (as plasma GPx; ref. 20), making our observation consistent with predictions.

Furthermore, the mean selenium concentration in our study was below the bottom of the range of plasma selenium (84–131 μg/L) in the study in which Li and colleagues (13) reported an association between the SOD2-Ala16Val genotype and prostate cancer risk when subjects were divided according to the quartile of selenium status. In that study, high selenium status was advantageous for SOD2-Ala16Val homozygotes as the combination of high SOD2 activity in the mitochondrion, together with high selenium/GPx, enabled the efficient removal of both reactive oxygen species, superoxide and H2O2. By contrast, Ala16 homoyzygotes were at increased risk, particularly of aggressive prostate cancer, when their selenium status was in the bottom quartile (13). Although we saw an overall effect of the SOD2-Ala16Val genotype in our low-selenium population, Li and colleagues (13) observed no overall effect, perhaps because the range of selenium status in their population encompassed both positive and negative effects of SOD2 genotype on prostate cancer risk leading to a null effect overall. Our findings might help explain why the SOD2-Ala16Val risk allele seems to vary from study to study in a number of cancers, and illustrates a gene-nutrient interaction in which the effect of genotype on risk reverses with changes in the nutrient status of the population.

Results from the Alpha-Tocopherol, Beta-Carotene (ATBC) study may further illustrate this point. Finnish smokers who were SOD2-Ala16 Val homozygotes had a 70% increased risk of prostate cancer when compared with Val homozygotes (28). This is consistent with the findings of Li and colleagues in their lowest selenium quartile in which the SOD-Ala16 homozygotes had an increased risk of prostate cancer (13) and suggests that men from the ATBC cohort must have had a similar selenium status to the bottom quartile of men from the U.S. Although at first sight, this might seem surprising because the cohort was recruited after the introduction of selenium-enriched fertilizers in Finland, in fact, there are data to show that the ATBC study did indeed include men with very low selenium status (29). When the use of selenized fertilizers was implemented in 1984, mean plasma selenium in Finland was

Table 4. The interaction between SOD2-Val16Ala and SEPP1-Ala234Thr SNPs in determining prostate cancer risk in never-smokers and ever-smokers in the CAPS study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Never-smokers</th>
<th></th>
<th>Ever-smokers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n)</td>
<td>Cases (n)</td>
<td>OR (95% CI)*</td>
<td>P</td>
</tr>
<tr>
<td>SEPP1 codon 234 Ala/Ala</td>
<td>142</td>
<td>252</td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>SOD2-Val/Val</td>
<td>38</td>
<td>51</td>
<td>Referent 1</td>
<td></td>
</tr>
<tr>
<td>SOD2-Ala/Val + Ala/Ala</td>
<td>104</td>
<td>201</td>
<td>1.44 (0.89–2.34) 0.14</td>
<td>152</td>
</tr>
<tr>
<td>SEPP1 codon 234 Ala/Thr + Thr/Thr</td>
<td>111</td>
<td>189</td>
<td></td>
<td>167</td>
</tr>
<tr>
<td>SOD2-Val/Val</td>
<td>27</td>
<td>43</td>
<td>Referent 1</td>
<td>39</td>
</tr>
<tr>
<td>SOD2-Ala/Val + Ala/Ala</td>
<td>84</td>
<td>146</td>
<td>1.08 (0.62–1.87) 0.80</td>
<td>128</td>
</tr>
<tr>
<td>P for interaction</td>
<td></td>
<td></td>
<td></td>
<td>0.0014</td>
</tr>
</tbody>
</table>

NOTE: n = number of subjects.
*ORs adjusted for age and geographic location, 95% CI.
Optimizing the selenium intake of individuals homozygous for SEPP1-Ala234 will improve their ability to supply selenium via selenoprotein P concentration were only observed under relatively low-selenium status are consistent with those findings. 

We found evidence for a gene-gene interaction between the Ala234Thr polymorphism in the selenium transport protein, SEPP1, and the SOD2-Val16Ala polymorphism. Individuals homozygous for SEPP1-Ala234 and SOD2-Ala16+ were 43% more likely to have prostate cancer compared with Val16 homozygotes (Table 2); this interaction was stronger in aggressive disease, resulting in a 60% increased risk. Prospective cohort studies have shown a stronger association between selenium status and risk of aggressive disease compared with localized disease (31–35).

Of relevance to our findings, Méplan and colleagues recently found that male SEPP1-Ala234 homozygotes had lower plasma selenoprotein P levels compared with heterozygotes, with an associated trend for reduced activity or protein concentration of GPx1 (cytosolic GPxs) and GPx4 (phospholipid GPxs; ref. 12). The authors suggest that the Ala234Thr polymorphism affects the stability of selenoprotein P, possibly through a posttranslational modification, affecting protein levels only when selenium intake is suboptimal. Thus, genotype-dependent differences in plasma selenoprotein P concentration were only observed under relatively low selenium conditions and disappeared after supplementation with selenium (12). Our observations in a population of relatively low-selenium status are consistent with those findings.

Because there is strong evidence that selenoprotein P plays a critical role in the delivery of hepatic selenium to other tissues (8, 9, 36), we hypothesize that men homozygous for the SEPP1-Ala234 allele have a reduced availability of selenium for the production of GPx in the prostate. This would impede the ability of the prostate to remove H₂O₂ and would therefore increase the risk of prostate cancer (refs. 24, 25; Fig. 2). In our study, this polymorphism only affected prostate cancer risk in conjunction with the SOD2-Ala16 allele, which, by allowing more efficient transport of superoxide dismutase into the mitochondrion, caused increased production of H₂O₂ that became detrimental in the face of low selenium. The gene-gene interaction between the SEPP1-Ala234Thr and SOD2-Val16Ala polymorphisms was also apparent in current or ex-smokers who had a 2-fold increased risk of prostate cancer compared with SOD2-Val16 homozygotes (Table 4). As we have postulated that the mechanism by which these polymorphisms have their combined effect is oxidative stress–related, the greater strength of the interaction in smokers than in the study as a whole, despite smaller numbers, might be explained by an exacerbation of oxidative stress in ever-smokers, although it may equally well be related to the lower selenium status seen in smokers (37, 38). In fact, we did not observe lower selenium status among smokers in our study but this may have been due to the small number (n = 169) of plasma selenium measurements made.

Our study has limitations in that HapMap shows considerable linkage disequilibrium at both these gene loci, therefore, we cannot be sure that these polymorphisms are the only functional SNPs affecting the risk of prostate cancer in the SOD2 and SEPP1 genes. However, given the published studies implying functional consequences of the amino acid changes, it would seem plausible that they have some function in this context. Both of these SNPs were included in the recent genomewide screen of a subset of our case-control study (498 aggressive cases and 494 controls), and results are consistent with the present data on a larger sample (39). Other recent genomewide SNP screens in prostate cancer have not implicated either of these genes (40–42), but the U.K. study did find a disease-associated SNP (rs9364554) in the SLC22A3 gene (40) on chromosome 6, just over 700 kb telomeric of SOD2. Analysis of HapMap CEU data reveals no linkage disequilibrium between this SNP and SOD2-Val16Ala, suggesting that the SOD2 SNP is independently associated with prostate cancer risk and is not acting as a marker for SLC22A3 association.

If confirmed, the evidence presented here would allow the identification of individuals who would particularly benefit from selenium supplementation to prevent prostate cancer. Among the Swedish men in our study, 41% had the high-risk genotype combination (homozygosity for SEPP1-Ala234 and possession of an SOD2-Ala16 allele). Similar allele frequencies have been identified in other populations of European descent (12, 13). We have observed the combined effect of the SOD2-Val16Ala and SEPP1-Ala234Thr SNPs under conditions of limited selenium availability. Such an effect can probably not be observed in areas of higher selenium status such as North America, where plentiful selenium supply can probably obviate the disadvantage of homozygosity for SEPP1-Ala234 except for those with the lowest intakes (13). Optimizing the selenium intake of individuals homozygous for SEPP1-Ala234 will improve their ability to supply selenium via the selenoprotein P pathway.
seleoprotein P for GPx synthesis and H$_2$O$_2$ removal (12). Confirmation of our findings in other populations with low selenium intake should be a high priority. Thus far, few genetic tests have had the potential to ameliorate risk by identifying the need for a simple, inexpensive, nutritional supplement.

Disclosure of Potential Conflicts of Interest

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