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# Manganese superoxide dismutase (MnSOD) gene polymorphism, interactions with carotenoid levels and prostate cancer risk

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***Background:*** **The manganese superoxide dismutase (*MnSOD*) gene encodes an antioxidant enzyme (SOD2) that may protect cells from oxidative damage. The MnSOD allele with *Val* as amino acid 16 encodes a protein that has 30–40% lower activity compared with the *MnSOD Ala* variant, hence possibly increasing susceptibility to oxidative stress. On the other hand, some epidemiologic studies suggest that the *Ala* allele is associated with a higher risk of cancer, including prostate cancer. *Methods:* We conducted a nested case–control study in the Health Professionals Follow-up Study with 612 incident prostate cancer cases and 612 matched controls to investigate the role of the *MnSOD* gene *Ala16Val* polymorphism and its joint association with plasma carotenoid concentrations in relation to risk of total prostate cancer and aggressive prostate cancer (advanced stage or Gleason sum ≥7). *Results:* The allele frequencies in the controls were 49.8% for *Ala* and 50.2% for *Val*. No association was found between the *MnSOD* genotype and risk of total and aggressive prostate cancer. Furthermore, no statistically significant interaction was observed between the *MnSOD* genotype and any of the plasma carotenoids in relation to risk of total and aggressive prostate cancer. In analyses in which we combined data from plasma and dietary carotenoids and created a quintile score to reflect long-term carotenoid status, a 3-fold [95% confidence interval: 1.37–7.02] increased risk of aggressive prostate cancer was observed among men with the *Ala/Ala* genotype in the presence of low long-term lycopene status (*P*-value, test for interaction = 0.02) as compared with men with the *Ala/Val+Val/Val* genotypes with low long-term lycopene status. *Conclusion:* In this cohort of mainly white men, the *MnSOD* gene *Ala16Val* polymorphism was not associated with total or aggressive prostate cancer risk. However, men with the *MnSOD Ala/Ala* genotype who had low long-term lycopene status had a higher risk of aggressive prostate cancer compared with individuals with the other genotypes. These results are consistent with findings from earlier studies that reported when antioxidant status is low, the *MnSOD Ala/Ala* genotype may be associated with an increased risk of aggressive prostate cancer.**

## Introduction

Endogenous antioxidant enzymes such as superoxide dismutase enzymes (SOD1 and SOD2) (1) together with exogenous antioxidants such as carotenoids are involved in the host defense mechanisms against reactive oxygen species and are hypothesized to reduce cancer risk (2). Manganese superoxide dismutase (SOD2 or MnSOD) is among the endogenous antioxidant enzymes in the pathway that converts reactive oxygen species to hydrogen peroxide ($H_2O_2$), which is ultimately converted into water by catalase and glutathione peroxidase (3–5). *MnSOD* is a single-copy gene with five exons and four introns (6). In many tumors, the region in chromosome 6q25.3, where MnSOD is located, is deleted, implicating MnSOD as a candidate tumor suppressor gene (7–10). Malignant prostate epithelium has lower *MnSOD* expression than benign prostatic epithelium (3) and *in vivo*, overexpression of MnSOD in the prostate is shown to inhibit cancer cell growth (9,10). Furthermore, MnSOD was shown to affect the proliferation of androgen-independent human prostate cancer (PC-3) cells *in vitro* and *in vivo* by retarding $G_1$ to S transition in the cell cycle and by regulating cell survival (11,12).

Localization of MnSOD into the mitochondrial matrix is essential for it to protect cells from oxidation (13,14). A cytosine to thymine ($C \rightarrow T$) single-nucleotide polymorphism (SNP) in the *MnSOD* gene at nucleotide 47 results in an alanine (*GCT*) to valine (*GTT*) change at the 9th position (16th amino acid from the beginning of the signal sequence) and can disrupt the α-helix structure of the MnSOD enzyme (rs4880) (15). The *Val* allele encodes a protein that is maintained at the mitochondrial inner membrane level and as a result has 30–40% less activity compared with the *Ala* allele (15). In addition, the *Val* allele may be associated with decreased messenger RNA stability (16,17).

In contrast to the hypothesis that the *Val* allele might be a high-risk allele, findings from a study among heavy smokers in Finland suggest that the *Ala* allele is the high-risk allele for prostate cancer (18). In the Physicians' Health Study (PHS), men with the *Ala/Ala* genotype were also found to be most susceptible to prostate cancer, but only if they had a low plasma antioxidant status (a sum of plasma lycopene, selenium and α-tocopherol) (19). In contrast, among men with high antioxidant status, possession of *Ala/Ala* genotype was associated with reduced risk. In a cohort study, men with the *Ala* allele appeared to be at a slightly higher risk of prostate cancer, especially if they smoked and had low vitamin E intake (20). However, in another cohort of heavy smokers, MnSOD variants were not associated with prostate cancer risk (21).

Thus, in the Health Professionals Follow-Up Study (HPFS), we prospectively investigated whether the *MnSOD* gene *Ala16Val* polymorphism was associated with prostate cancer risk. We further investigated whether the *MnSOD* genotype coupled with low plasma carotenoid or with low long-term carotenoid status was associated with increased prostate cancer risk. We were particularly interested in interactions with lycopene status, which was associated with reduced risk of prostate cancer in this cohort and some, though not in all others (22–24).

## Materials and methods

### *Study design and study population*

We conducted a nested case–control study within the HPFS, a cohort of 51 529 USA predominantly white (97%) male health professionals, that began in 1986. Eligible for inclusion as cases in the present study were all men who were diagnosed with prostate cancer prior to February 2000 and after returning a blood sample in 1993–1995. Reasons for exclusion from the baseline cohort and detailed descriptions of HPFS blood collection procedures have been reported elsewhere (23). Cases were excluded if they were diagnosed with any other cancer except non-melanoma skin cancer prior to the date of blood draw. Additionally, indolent microscopic focal tumors (T1a) found incidentally during transurethral resection of the prostate were excluded in order to minimize detection bias.

**Abbreviations:** CI, confidence interval; $H_2O_2$, hydrogen peroxide; HPFS, Health Professionals Follow-Up Study; MnSOD or SOD2, manganese superoxide dismutase; PHS, Physicians' Health Study; PSA, prostate-specific antigen; QC, quality control; RR, relative risk; SNP, single-nucleotide polymorphism.

 

Cases were ascertained by the participant (or next of kin for decedents) reporting a diagnosis of prostate cancer or from death certificates if next of kin could not be contacted. The study investigators, upon receiving permission, successfully obtained medical and pathology records for 90% of the cases in the cohort. The remainder of the cases was self-reported. Seven hundred and four (98%) total prostate cancer cases detected mainly by a prostate-specific antigen (PSA) test were diagnosed, between the start of follow-up (date of blood return) and January 2000. Controls were eligible for selection if they were still alive and if they had a PSA test following the blood draw and remained free of prostate cancer at the time of the case's diagnosis. One control was matched to one case on year of birth and year of blood return, history of a PSA test before blood draw (yes/no), time of day at blood draw and season.

We classified prostate cancer by stage using the 1992 version of the American Joint Committee on Cancer tumor-nodes-metastasis (TNM) staging manual (25) and by grade using Gleason sum at diagnosis. 'Early stage' cases were those that were apparently organ confined with no involvement of the seminal vesicle (T1 or T2 or T3a and N0M0 stage), whereas 'advanced-stage' cases were those with local spread to the seminal vesicle and beyond (T3c or T4 and N0M0, any T and N (1–3) or any T and M1 stage). Tumors with Gleason sum $<7$ were classified as 'well to moderately well differentiated', whereas those with a Gleason sum $\geq 7$ were classified as 'poorly differentiated'. Finally, tumor aggressiveness was defined based on a combination of stage and Gleason grade: the apparently organ-confined tumors and Gleason sum $<7$ were defined as 'less aggressive'; advanced stage or Gleason sum $\geq 7$ was defined as 'more aggressive' tumors.

*Nutrient intake assessment.* To estimate nutrient intake, we used a 131-food item semi-quantitative food frequency questionnaire first administered in 1986 and then every 4 years thereafter (26). For each item, the respondent indicates average intake over the past year of a standard portion size. The nutrient contribution by each food or beverage item was assessed by multiplying the frequency that the item was consumed by the nutrient content of its serving. Then, the nutrient intakes were calculated by summing the nutrient contributions from all food and beverage items. To estimate baseline nutrient intake levels, the 1994 food frequency questionnaire, the questionnaire administered closest to the time of blood drawn between 1993 and 1995, was used. To capture the long-term cumulative average carotenoid intake, the average of nutrient intakes from the 1986, 1990 and 1994 food frequency questionnaire was used for all cases and controls (27).

*Laboratory analyses*

*Carotenoid assays.* To measure the concentrations of carotenoids in the study population, plasma samples of the matched case–control pairs were placed next to each other in boxes in random order and sent on dry ice to the laboratory of John W. Erdman (Department of Food Science and Nutrition, University of Illinois). Case–control pairs were assayed blindly in the same batch. Replicates from two pools of plasma were used to make the quality control (QC) samples and were inserted randomly in pairs throughout the boxes. Laboratory technicians were blinded for the QC status of the samples. A detailed description of serum preparation, serum extraction and high-performance liquid chromatography analysis has been described elsewhere (23). The gradient reverse phase high-performance liquid chromatography method utilized was modified from Yeum *et al.* (28) as described by Boileau *et al.* (29).

Carotenoids were processed in three batches: the first batch (1996) included cases diagnosed from 1993 through January 1996 (and their matched controls); the second batch (1998) included cases diagnosed from February 1996 through January 1998 and the third batch (2000) included cases diagnosed from February 1998 through January 2000. The plasma carotenoids' batch-specific (1996, 1998 and 2000) mean intrapair coefficients of variation (CV %) were calculated and the overall range was from 1.3 to 12.3%, suggesting acceptable within assay measurement error (8.1–12.3% for lutein, 4.6–9.9% for β-cryptoxanthin, 1.3–11.3% for α-carotene, 4.7–6.7% for β-carotene and 5.2–11.9% for total lycopene). Although some of the samples had been stored for up to 10 years before the carotenoid assays were performed, the samples were stored at highly stable conditions in liquid nitrogen. Furthermore, significant degradation was unlikely because in a sample of 144 men, the correlation of plasma lycopene from samples taken 3–4 years apart was high ($r = 0.63$; $P < 0.001$) (30). Cholesterol concentrations were measured in the laboratory of Dr Steven Clinton using the Infinity Total Cholesterol enzymatic assay kit (Sigma Diagnostics, St Louis, MO) as per manufacturer's recommendations.

*Genotyping methods.* Genomic DNA was extracted in 96-well plate format using the Qiagen QIAamp blood kit protocol (Qiagen, Chatsworth, CA) after diluting 50 µl of buffy coat with 150 µl of phosphate-buffered saline. A random 10% of the samples were included as duplicates for QC purposes. The results across the duplicate samples were assessed for consistency and were 100% concordant. Cases and their matched controls were genotyped in 384-well format using the TaqMan technology (Applied Biosystems, Foster City, CA) (31). Genotyping was always done for cases and their matched controls in the same analytical run with no identifiers for laboratory personnel for case, control or QC status. Primer and probe sequences for polymerase chain reaction amplification are available from the authors on request.

*Statistical analysis*

*MnSOD genotype.* Utilizing the $\chi^2$ test (32), we evaluated Hardy–Weinberg equilibrium by comparing the observed genotype frequencies to the expected genotype frequencies among controls. We created indicator variables for genotypes (*Ala/Val* and *Ala/Ala*) for comparison with the reference *Val/Val* genotype. Under the recessive inheritance model assumption, we created a dichotomous variable by collapsing the *Val/Val* and *Val/Ala* genotypes into a reference group since most reports of the *MnSOD* SNP and cancer association had identified the *Ala/Ala* genotype as the high-risk genotype (18,19,33). We used univariate and multivariate conditional logistic regression models to estimate relative risk (RR) and their 95% confidence intervals (CIs). To maximize power for evaluating associations by stage and grade, we dropped the matching to include all controls in the analysis; we then used unconditional logistic regression models adjusting for the matching factors.

Initially, 1408 participants (704 matched case–control pairs) were selected. Thirty-six participants were excluded for the following reasons: diagnosis of prostate cancer was determined to be prior to the date of blood draw or the date of diagnosis was missing for the case or control in 8 pairs; plasma was insufficient or plasma measurements were missing for the case or control in 28 pairs. For analyses for which genotype was the main exposure, we further deleted the 78 participants with missing genotype for either the case or the control, so the final number of available participants was 1294.

In addition to taking into account the matching factors, we included in the multivariate models first-degree family history of prostate cancer, region of residence, cigarette smoking status, plasma 25(OH)vitamin D and plasma $1,25(OH)_2$vitamin D, current vitamin E supplement use, current selenium supplement use, plasma cholesterol, energy intake, body mass index (kg/m$^2$), fish, average daily intake of total calcium, total alcohol and α-linolenic acid. Average daily intake of tomato sauce was included in the multivariate models when the main exposure was not plasma total lycopene or plasma total carotenoid. Plasma carotenoids are transported in the blood by lipoproteins and so we also included plasma cholesterol in the multivariate models to reduce extraneous variation from cholesterol level (34).

*Plasma and dietary carotenoids.* A variable for total plasma carotenoids was created by summing the individual concentrations of plasma lutein/zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and total lycopene. The distribution of individual and total plasma carotenoids between the matched cases–control pairs was compared, and the statistical significance of any differences observed was assessed with the Wilcoxon signed rank test. Quintiles of plasma carotenoids were created with the batch-specific quintile cutoffs obtained from the controls in each batch. Low plasma carotenoid was also defined as below the batch-specific median cut points among the controls to offset batch-to-batch variability for each plasma carotenoid. Finally, to investigate the effect of long-term carotenoid status, we created a plasma/diet score variable as described previously (23) by summing deciles of plasma and dietary intake of carotenoids (based on the cumulative average updated dietary intake to 1994) and then making quintiles based on the combined score. By combining these two sources of information on carotenoids, our aim was to attenuate the misclassification of long-term exposure (35–37). Tests for trend were performed, with levels of plasma carotenoids or plasma/diet score entered as single ordinal variables with values of 1–5 corresponding to categories created using the batch-specific quintile cut points from the controls.

*Evaluation of gene–environment interaction.* To determine the joint association of the MnSOD genotype and carotenoid level on prostate cancer risk, we created multivariate models that included each level of genotype cross-classified with each level of carotenoids (below versus at or above the median or as quintiles). To test the hypothesis that the influence of low plasma carotenoids or low long-term carotenoid status on prostate cancer risk is modified by increasing copies of the *Ala* allele, a likelihood ratio test was conducted where a natural order was assumed for the number of copies of the *Ala* allele. Here, we compared the model with the main effect of the *MnSOD* genotype entered as an ordinal variable coded as 1–3 and the main effect of plasma carotenoid entered as a dichotomous variable (below versus at or above the median) or the main effect of long-term carotenoid status entered as an ordinal plasma/diet score variables coded as 1–5, to a model that included the main effect terms and the interaction term, which was the product of the main effect terms. In secondary analyses, the role of the MnSOD genotype as a modifier of exposures that may influence oxidative stress, cigarette smoking and alcohol consumption in relation to prostate cancer risk was evaluated.

## Results

*MnSOD genotype*

The mean ± standard deviation age at blood draw for both cases and age-matched controls was 66 ± 7 years. Mean time between blood draw and prostate cancer diagnosis was 3.1 ± 1.7 years. Of the 92.1% of cases for whom information was available on stage, 9.0% were advanced, and of the 90.2% cases for whom information was available on grade, 38.7% were poorly differentiated and 42.1% were aggressive (poorly differentiated or advanced stage) prostate cancers. The allele frequencies in the controls were 49.8% for *Ala* and 50.2% for *Val*, similar to what was reported previously in Caucasians (33). The HPFS cohort is predominantly a white population (~1% African-American and 2% Asian-American). The control group was in Hardy–Weinberg equilibrium for the *MnSOD Ala* to *Val* SNP ($P$ = 0.69). No association was observed between the *MnSOD* genotypes and prostate cancer overall or by stage or grade, although a slight but non-statistically significant increase risk of poorly differentiated and aggressive prostate cancer was observed for the *Ala/Ala* genotype (Table I).

*Plasma and dietary carotenoids*

Among total or aggressive prostate cancer cases and their matched controls, no significant differences were seen in median plasma levels for lutein/zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, total lycopene and total carotenoids (supplementary Table 1 is available at *Carcinogenesis* Online). No statistically significant evidence of a linear relationship was found between any of the plasma carotenoids and total prostate cancer risk or for long-term carotenoid status and prostate cancer risk overall or by age (supplementary Table 2 is available at *Carcinogenesis* Online). These results were null both when plasma/diet scores were based on the cumulative updated averages between 1986 and 1994 and when based on the baseline 1994 diet data.

*Interactions between MnSOD, lycopene and other carotenoids*

No statistically significant interaction was found between the *MnSOD* genotype and any specific plasma carotenoid or plasma total carotenoids in relation to risk of total prostate cancer overall (Table II) or aggressive prostate cancer (Table III). However, a suggestive but not statistically significant interaction ($P$ = 0.10) was seen for plasma lycopene and aggressive prostate cancer; among men with lycopene levels above the median, no association was observed for *MnSOD* genotype, but among men with low plasma lycopene, those with the *Ala/Ala* genotype had approximately a 2-fold elevated risk compared with those with the *Val/Val* genotype (Table III). A similar interaction was observed for those with poorly differentiated prostate cancer ($P$ = 0.09), which comprised the majority of aggressive prostate cancer (data not shown).

We then examined the joint effect of *MnSOD* genotype and long-term carotenoid status (based on the plasma and carotenoid intake combined measure). A multiplicative interaction was suggested between the *MnSOD* genotype and long-term lycopene status in relation to total prostate cancer risk. Men with the *Ala/Ala* genotype had a reduced total prostate cancer risk, which was marginally statistically significant ($P$-value, test for interaction = 0.07), in the presence of high lycopene status compared with men with the *Ala/Val* and *Val/Val* genotypes in the presence of low lycopene status (RR = 0.54, 95% CI: 0.27–1.10) (Figure 1). Among men with the *Ala/Ala* genotype, those in the low quintile of lycopene score had an RR = 2.44, 95% CI: 1.02–5.88 relative to those in the high quintile ($P$-value, test for trend = 0.01). Furthermore, for aggressive prostate cancer, among men in the lowest quintile of lycopene status, men with the *Ala/Ala* genotype were found to have a 3-fold greater risk (RR = 3.10, 95% CI: 1.37–7.02; $P$-value, test for interaction = 0.02) compared with men with the *Ala/Val* and *Val/Val* genotypes (Figure 2).

*Interactions between MnSOD and lifestyle factors influencing oxidative stress*

No statistically significant interactions between the *MnSOD* genotype and cigarette smoking or alcohol consumption were found in relation to total prostate cancer or by stage or grade (data not shown).

## Discussion

Overall, in the HPFS, we found no evidence of an association between the *MnSOD* genotype and prostate cancer risk. In relation to total and aggressive prostate cancer risks, we observed an interaction between

**Table I.** Association of MnSOD polymorphism and risk of prostate cancer in the HPFS, 1993–2000

| Genotype | Cases | | Controls | | RR (95% CI)[a] | RR (95% CI)[b] |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| Total prostate cancer | | | | | | |
| Val/Val (ref) | 156 | 24.3 | 162 | 24.9 | 1.00 (ref) | 1.00 (ref) |
| Val/Ala | 320 | 49.8 | 331 | 50.8 | 1.03 (0.78–1.37) | 1.11 (0.82–1.49) |
| Ala/Ala | 166 | 25.9 | 159 | 24.4 | 1.11 (0.80–1.53) | 1.12 (0.80–1.57) |
| Poorly differentiated prostate cancer[c] | | | | | | |
| Val/Val (ref) | 55 | 24.6 | 162 | 24.9 | 1.00 (ref) | 1.00 (ref) |
| Val/Ala | 101 | 45.1 | 331 | 50.8 | 0.90 (0.61–1.32) | 0.96 (0.65–1.43) |
| Ala/Ala | 68 | 30.4 | 159 | 24.4 | 1.28 (0.84–1.95) | 1.37 (0.89–2.12) |
| Well-differentiated prostate cancer[c] | | | | | | |
| Val/Val (ref) | 83 | 23.4 | 162 | 24.9 | 1.00 (ref) | 1.00 (ref) |
| Val/Ala | 193 | 54.4 | 331 | 50.8 | 1.11 (0.81–1.54) | 1.14 (0.81–1.59) |
| Ala/Ala | 79 | 22.3 | 159 | 24.4 | 0.99 (0.67–1.44) | 0.99 (0.67–1.47) |
| Aggressive prostate cancer[d] | | | | | | |
| Val/Val (ref) | 61 | 25.0 | 162 | 24.9 | 1.00 (ref) | 1.00 (ref) |
| Val/Ala | 111 | 45.5 | 331 | 50.8 | 0.89 (0.62–1.29) | 0.94 (0.64–1.37) |
| Ala/Ala | 72 | 29.5 | 159 | 24.4 | 1.20 (0.80–1.81) | 1.26 (0.83–1.92) |

[a]Estimated from conditional logistic regression model. Cases and controls were matched on year of birth (±1 year), history of a PSA test before blood draw, quadrant of day of blood draw (midnight to before 9 a.m., 9 a.m. to before noon, noon to before 4 p.m. and 4 p.m. to before midnight), season (spring, summer, fall and winter) and year of blood draw.
[b]Additionally adjusted for family history, region of residence, fish intake, smoking status, current vitamin E supplement use, current selenium supplement use, total calcium intake, plasma 25(OH)D and plasma $1,25(OH)_2D$, body mass index, total alcohol, energy and tomato sauce intake.
[c]Gleason sum ≥7 is poorly differentiated and Gleason sum <7 is well differentiated.
[d]Tumors with T3c or T4 and N0M0, any T and N (1–3), any T and M1 stage or Gleason grade ≥7.

**Table II.** Joint association of MnSOD *Ala16Val* genotypes and plasma carotenoids[a] with total prostate cancer

| MnSOD Plasma carotenoid | Val/Val OR (95% CI) | Val/Ala OR (95% CI) | Ala/Ala OR (95% CI) | *P*-value, test for interaction[b] |
|---|---|---|---|---|
| Lutein/zeaxanthin (nmol/l)[c] | | | | 0.62 |
| Above median | 1.00 (ref) | 1.01 (0.61–1.67) | 1.19 (0.70–2.02) | |
| Below median | 1.07 (0.61–1.86) | 1.25 (0.77–2.02) | 1.04 (0.59–1.84) | |
| Beta-cryptoxanthin (nmol/l)[c] | | | | 0.97 |
| Above median | 1.00 (ref) | 1.16 (0.72–1.88) | 1.08 (0.64–1.82) | |
| Below median | 1.03 (0.58–1.81) | 1.08 (0.67–1.76) | 1.16 (0.66–2.01) | |
| Alpha-carotene (nmol/l)[c] | | | | 0.68 |
| Above median | 1.00 (ref) | 1.17 (0.74–1.87) | 1.20 (0.70–2.06) | |
| Below median | 0.97 (0.58–1.65) | 1.02 (0.64–1.63) | 1.00 (0.60–1.67) | |
| Beta-carotene (nmol/l)[c] | | | | 0.62 |
| Above median | 1.00 (ref) | 1.16 (0.72–1.89) | 1.21 (0.71–2.06) | |
| Below median | 1.19 (0.68–2.08) | 1.25 (0.77–2.01) | 1.19 (0.68–2.07) | |
| Total lycopene (nmol/l)[c] | | | | 0.26 |
| Above median | 1.00 (ref) | 1.10 (0.68–1.77) | 0.91 (0.53–1.54) | |
| Below median | 0.93 (0.53–1.62) | 1.08 (0.67–1.73) | 1.27 (0.74–2.17) | |
| Total carotenoid (nmol/l)[c] | | | | 0.70 |
| Above median | 1.00 (ref) | 1.22 (0.76–1.97) | 1.02 (0.60–1.75) | |
| Below median | 1.12 (0.66–1.91) | 1.17 (0.73–1.88) | 1.32 (0.78–2.23) | |

OR, odds ratio.
[a]According to the batch-specific median cutoffs of each plasma carotenoid among controls.
[b]The likelihood ratio test was used to test the statistical significance of the interaction terms between plasma carotenoid and MnSOD genotype, where a natural order was assumed for genotype.
[c]Estimated from conditional logistic regression model. Cases and controls were matched on year of birth (±1 year), history of a PSA test before blood draw, quadrant of day of blood draw (midnight to before 9 a.m., 9 a.m. to before noon, noon to before 4 p.m. and 4 p.m. to before midnight), season (spring, summer, fall and winter) and year of blood draw and additionally adjusted for family history, region of residence, fish intake, plasma cholesterol, smoking status, current vitamin E supplement use, current selenium supplement use, total calcium intake, plasma 25(OH)D and plasma $1,25(OH)_2D$ (dichotomized as deficient/not deficient), body mass index, total alcohol, energy and tomato sauce intake. Note: tomato sauce was not adjusted in models with total plasma lycopene and total plasma carotenoid as main exposures.

**Table III.** Effect modification of the relationship between plasma carotenoids[a] and aggressive prostate cancer[b] by the MnSOD genotypes

| MnSOD | Val/Val OR (95% CI) | Val/Ala OR (95% CI) | Ala/Ala OR (95% CI) | *P* interaction[c] |
|---|---|---|---|---|
| Plasma lutein/zeaxanthin (nmol/l)[d] | | | | 0.18 |
| Above median | 1.00 (ref) | 0.90 (0.49–1.66) | 1.65 (0.87–3.12) | |
| Below median | 1.09 (0.55–2.12) | 1.14 (0.63–2.06) | 1.02 (0.50–2.07) | |
| Plasma β-cryptoxanthin (nmol/l)[d] | | | | 0.86 |
| Above median | 1.00 (ref) | 1.00 (0.56–1.79) | 1.31 (0.69–2.52) | |
| Below median | 1.13 (0.57–2.22) | 1.07 (0.60–1.91) | 1.44 (0.75–2.77) | |
| Plasma α-carotene (nmol/l)[d] | | | | 0.38 |
| Above median | 1.00 (ref) | 1.05 (0.59–1.88) | 1.60 (0.84–3.05) | |
| Below median | 1.16 (0.59–2.27) | 1.05 (0.58–1.88) | 1.23 (0.64–2.37) | |
| Plasma β-carotene (nmol/l)[d] | | | | 0.91 |
| Above median | 1.00 (ref) | 1.05 (0.58–1.89) | 1.27 (0.66–2.46) | |
| Below median | 1.26 (0.64–2.47) | 1.15 (0.64–2.08) | 1.66 (0.87–3.17) | |
| Plasma total lycopene (nmol/l)[d] | | | | 0.10 |
| Above median | 1.00 (ref) | 0.84 (0.48–1.48) | 0.90 (0.47–1.71) | |
| Below median | 0.77 (0.40–1.50) | 0.90 (0.52–1.59) | 1.47 (0.78–2.75) | |
| Plasma total carotenoid (nmol/l)[d] | | | | 0.65 |
| Above median | 1.00 (ref) | 0.98 (0.56–1.72) | 1.19 (0.63–2.25) | |
| Below median | 0.95 (0.49–1.85) | 0.95 (0.54–1.67) | 1.36 (0.73–2.54) | |

OR, odds ratio.
[a]According to the batch-specific median cutoffs for plasma carotenoids.
[b]Tumors with T3c or T4 and N0M0, any T N (1–3), and any T M1 stage or Gleason grade $\geq$7.
[c]The likelihood ratio test was used to test the statistical significance of the interaction terms between plasma carotenoid and MnSOD genotype, where a natural order was assumed for genotype.
[d]Estimated from conditional logistic regression model. See Table II for covariates.

*MnSOD* genotype and the score variable created by summing plasma and dietary lycopene levels to reflect long-term lycopene status. Men with the *MnSOD Ala/Ala* genotype were found to be more susceptible to total and particularly aggressive prostate cancer in the presence of low long-term lycopene status.

These results were similar to those from the PHS cohort, another population of mostly white health professionals in the USA (19) in suggesting an interaction with antioxidant intake or status. In the PHS, a study of 567 prostate cancer cases and 764 controls nested in a double-blind randomized clinical trial for β-carotene (19); men with the *Ala/Ala*

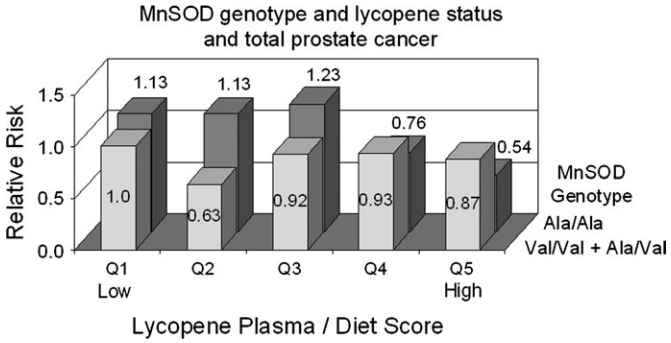


**Fig. 1.** The likelihood ratio test was used to test the statistical significance of the interaction terms between the MnSOD genotype and the plasma/diet carotenoid score variable, where a natural order was assumed for the plasma/diet carotenoid score variable (quintile). $P$ = value, test for interaction = 0.07.

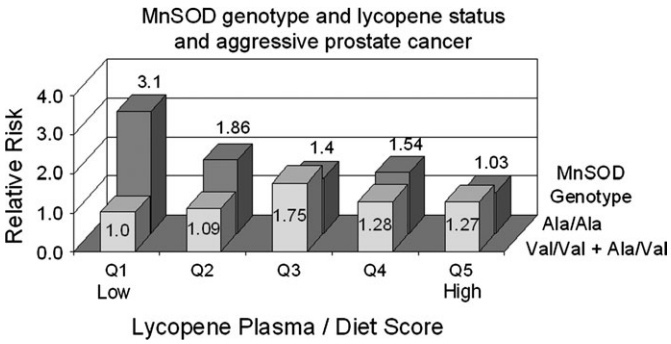


**Fig. 2.** The likelihood ratio test was used to test the statistical significance of the interaction terms between the MnSOD genotype and the plasma/diet carotenoid score variable, where a natural order was assumed for the plasma/diet carotenoid score variable (quintile). *P*-value, test for interaction = 0.02 (odds ratio = 3.1, 95% CI: 1.37–7.02).

genotype who were assigned to the β-carotene arm were at a 63% lower risk of fatal prostate cancer compared with men assigned to the placebo arm (RR = 0.37; 95% CI: 0.15–0.94; *P*-value, test for interaction = 0.03) (19). In addition, an interaction was observed in relation to both total (*P*-value, test for interaction = 0.02) and aggressive (*P*-value, test for interaction = 0.01) prostate cancers with a score variable that summed plasma antioxidant (lycopene, selenium and α-tocopherol) levels (19). Among men with the *Ala/Ala* genotype, high versus low plasma antioxidant levels were associated with a lower risk of total prostate cancer (RR = 0.20; 95% CI: 0.09–0.49) and aggressive prostate cancer (RR = 0.10; 95% CI: 0.03–0.29). For men with a *Val* allele, plasma antioxidant level was not associated with prostate cancer risk. Similarly, in another USA cohort (Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial), the Ala variant of *MnSOD* was associated with a moderately increased risk of prostate cancer primarily among men with lower intakes of dietary and supplemental vitamin E, particularly if they were smokers (20). However, in another USA cohort of heavy smokers, MnSOD variants were unrelated to risk, although interactions with antioxidant status were not assessed (21).

Although the findings for the main effect of *MnSOD* SNP in our study and the PHS and Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial studies were somewhat similar, they appear to differ from that of the Finnish alpha-tocopheral beta carotene (ATBC) study, in which the *MnSOD Ala/Ala* genotype was associated with an odds ratio of 1.72 (95% CI: 0.96–3.08, $P$ = 0.07) of total prostate cancer and an odds ratio of 2.72 (95% CI: 1.15–6.40, $P$ = 0.02) of high-grade prostate cancer. All the men in the ATBC study were long-term smokers, in contrast to a very small proportion of smokers in the USA studies. Furthermore, lycopene intake is much lower in Finland than in the USA. Given their high smoking rate and the presumably low antioxidant intake, in the Finnish study, antioxidant status may have been poor for the population at large. In fact, the results from the HPFS, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial and PHS cohorts suggest that the overall effect of the *MnSOD Ala/Ala* genotype will vary by antioxidant status, being inversely associated with risk of prostate cancer (especially aggressive) in a 'rich' antioxidant environment while increasing risk in a 'poor' antioxidant environment. If this model is correct, the results from the Finnish study are compatible with the USA studies.

While the functions of the *MnSOD* SNP have not been completely elucidated, Li *et al.* (19) hypothesized a model to account for the opposing effects on risk depending on antioxidant status. MnSOD converts the superoxide anion into oxygen and $H_2O_2$. Those homozygous for the *Ala* allele have higher MnSOD expression compared with those with at least one *Val* allele and higher MnSOD expression may result in an environment high in $H_2O_2$ concentration (38). Glutathione peroxidase, which detoxifies the $H_2O_2$ into water, is dependent on selenium. If catalase or glutathione peroxidase enzyme activities are low, then high MnSOD expression may lead to $H_2O_2$ toxicity (39) and thus possibly increase susceptibility to prostate cancer (40). However, when antioxidant level is adequate, the increased rate of superoxide anion quenching related to the *Ala* allele may be beneficial. Thus, depending on antioxidant status, the *MnSOD Ala* allele may be beneficial or deleterious.

We extended our prior investigation of the association between plasma carotenoids and prostate cancer to include cases ascertained through early 2000, which increased the sample size by 26% (197 additional pairs) compared with our previous publication (23). In the prior analysis, we observed marginally significant inverse associations for plasma lycopene concentrations and long-term lycopene status with total prostate cancer (23). In the extended analysis, the results for lycopene were attenuated. The differences in findings suggest a role of chance or that perhaps the inverse association with lycopene diminishes as the time between measurement of the assay and diagnosis lengthens. Of note, in our recent analysis of the entire HPFS cohort of 3544 cases up to 2002, long-term high intake of tomato sauce (the main source of bioavailable lycopene) remained significantly associated with lower prostate cancer risk (39). This relatively modest (about a 20%) reduction in prostate cancer risk attributed to tomato sauce could have been easily missed in this subgroup with plasma levels available which comprised only 20% of the total prostate cancer cases in the HPFS.

Our findings on the gene and environment interactions are not necessarily generalizable to men of other ethnicity because the HPFS is a relatively homogenous white population (∼1% African-Americans and ∼2% Asian-Americans) and the *Ala* allele frequency differs across populations. Our study had reasonable power to address the hypothesis of interest with respect to the primary hypotheses in relation to total ($n$ = 642), aggressive ($n$ = 244), poorly differentiated ($n$ = 224) and well-differentiated ($n$ = 355) prostate cancers. Our variable for aggressive prostate cancer was driven mostly by poorly differentiated cancers (advanced cancers that were well differentiated or of unknown grade comprised only 8% of the aggressive cancers). With only 58 advanced prostate cancer cases, we had limited statistical power to investigate all but very strong associations. One of the strengths of our study was to ensure equal opportunity for cancer detection in cases and controls; only controls who had remained free of prostate cancer diagnosis after having gone through a PSA test following the blood draw were eligible for inclusion into our study. A limitation of our study is that we did not have plasma data on vitamin E or selenium.

In summary, while no statistically significant evidence for a main effect was found for the *MnSOD* genotype in this cohort, men with the *MnSOD Ala/Ala* genotype who had low lycopene status were found to have a higher risk of aggressive prostate cancer compared with individuals with the other *MnSOD* genotypes. These results confirm findings from earlier studies that reported when antioxidant status is low,

the *MnSOD Ala/Ala* genotype may be associated with an increased risk of aggressive prostate cancer. Larger studies are warranted to investigate the joint association between MnSOD genotype and levels of other sources of plasma and dietary antioxidants.

## Supplementary material

Supplementary Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

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