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Associations Between Circulating Carotenoids, Genomic Instability and the Risk of High-Grade Prostate Cancer

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Abstract

BACKGROUND—Carotenoids are a class of nutrients with antioxidant properties that have been purported to protect against cancer. However, the reported associations between carotenoids and prostate cancer have been heterogeneous and lacking data on interactions with nucleotide sequence variations and genomic biomarkers.

OBJECTIVE—To examine the associations between carotenoid levels and the risk of high-grade prostate cancer, also considering antioxidant-related genes and tumor instability.

METHODS—We measured plasma levels of carotenoids and genotyped 20 single nucleotide polymorphisms (SNP) in SOD1, SOD2, SOD3, XRCC1, and OGG1 among 559 men with non-metastatic prostate cancer undergoing radical prostatectomy. We performed copy number analysis in a subset of these men (n =67) to study tumor instability assessed as Fraction of the Genome Altered (FGA). We examined associations between carotenoids, genotypes, tumor instability and risk of high-grade prostate cancer (Gleason grade ≥4 +3) using logistic and linear regression.

RESULTS—Circulating carotenoid levels were inversely associated with the risk of high-grade prostate cancer; odds ratios (OR) and 95% confidence intervals (CI) comparing highest versus lowest quartiles were: 0.34 (95% CI: 0.18–0.66) for α-carotene, 0.31 (95% CI: 0.15–0.63) for β-carotene, 0.55 (0.28–1.08) for lycopene and 0.37 (0.18–0.75) for total carotenoids. SNPs rs25489

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Conflicts of interest: JM Chan is a shared owner of a non-commercialized patent to examine circulating antioxidant nutrients and MnSOD in relation to aggressive prostate cancer.

in XRCC1, rs699473 in SOD3 and rs1052133 in OGG1 modified these associations for α-carotene, β-carotene and lycopene, respectively ($P \leq 0.05$). The proportion of men with a high degree of FGA increased with Gleason Score ($P < 0.001$). Among men with Gleason score ≤3 +4, higher lycopene levels were associated with lower FGA ($P = 0.04$).

CONCLUSION—Circulating carotenoids at diagnosis, particularly among men carrying specific somatic variations, were inversely associated with risk of high-grade prostate cancer. In exploratory analyses, higher lycopene level was associated with less genomic instability among men with low-grade disease which is novel and supports the hypothesis that lycopene may inhibit progression of prostate cancer early in its natural history.

Keywords

prostate cancer; lycopene; carotenes; genomic instability; genetic polymorphisms

INTRODUCTION

Chromosomal damage and genomic instability are hallmarks of cancer. Genetics and micronutrients, nutrients required in small quantities for a range of physiological functions, interact to protect the normal human genome from damage that may result in cancer.

Prostate cancer often has a long natural history but is still the second leading cause of cancer mortality in American men, accounting for nearly 28,000 deaths yearly [1]. Evidence suggests that several antioxidant micronutrients may be protective against the development of prostate cancer. Dietary intake of carotenoids, and especially lycopene, have been extensively studied, mainly supporting a protective effect on prostate cancer development [2–8]. In spite of these results, null findings have also been reported [9–13]. Studies of the association between circulating levels of carotenoids in plasma and prostate cancer risk have generated similarly heterogenous results [14–20]. Generally, studies conducted before the widespread use of prostate-specific antigen (PSA) testing, and thus including more advanced cases, are supportive of a beneficial role of carotenoids in prostate cancer prevention. This has lead to the suggestion that carotenoids may specifically inhibit or deter prostate cancer progression, rather than its initiation [8,21]. Further, it has been reported that the association between plasma levels of antioxidants, including some carotenoids such as lycopene, and risk of prostate cancer may be modified by polymorphisms in genes involved in DNA repair and antioxidant metabolism, such as the MnSOD (Manganese Superoxide Dismutase, SOD2) gene [22,23].

The question of whether antioxidant status post diagnosis, that is, secondary prevention, can affect the clinical course of disease is an understudied area. Data suggests that consumption of specific micronutrients after diagnosis may influence prostate cancer outcomes [24]. Further, it has been shown that overall tumor genomic instability, measured by degree of DNA copy number variations, associates with aggressive prostate cancer [25]. In this study, we sought to expand on these findings in a synergistic fashion by investigating whether circulating carotenoid levels were associated with lower risk of presenting with prostate cancers of higher grade and/or levels of genomic instability, and whether these associations were modified by germline genotypes in antioxidant and DNA repair-related genes.

MATERIALS AND METHODS

Study Population

For this study, we identified a cohort of 700 men diagnosed with localized prostate cancer in 2000–2007 and underwent radical prostatectomy as their primary treatment without neo-adjuvant therapy at the University of California, San Francisco (UCSF) Helen Diller Comprehensive Cancer Center. Participants consented to clinical follow-up, donated blood immediately prior to surgery (median 3.6 months after diagnosis), and provided prostatectomy tissue for research purposes. We preferentially selected men with high risk prostate cancer defined as Gleason sum ≥ 8 or PSA level at diagnosis ≥20 ng/ml or T-stage ≥T3 to enable us to identify risk factors for aggressive disease; 32% of the men in our study population had high-risk disease versus 22% of the 1,003 potentially eligible men who underwent radical prostatectomy at UCSF between 2000 and 2007. For this analysis, 559 of the 700 had sufficient plasma available for analysis.

From the cohort of 700, we randomly selected 120 men, evenly split by low (i.e., Gleason sum <=6 and PSA <10 ng/ml and Stage T2/T2a) and high risk groups for a subset exploratory assessment of fraction of the genome altered using array comparative genomic hybridication (aCGH). Of these 120, 16 were rejected because of insufficient tumor upon pathology review (i.e., required >=70% tumor for aCGH analysis); 13 were not used because of suspicion of neo-adjuvant hormones and one because of high grade prostate intraepithelial neoplasia identified at pathologic review (both of which could affect the aCGH measure in unknown ways); and nine had poor quality or insufficient DNA. Thus, 81 participants had their tumors assessed successfully by aCGH, of whom 67 also had plasma for circulating carotenoids.

Thus, the final study population for this analysis included 559 men with data on circulating carotenoids and germline gene variants, 67 of whom also had data on FGA. This research was approved by the Institutional Review Board of the University of California, San Francisco.

Plasma Collection and Antioxidant Measurement

Plasma samples were collected in EDTA-tubes prior to radical prostatectomy for each participant. Plasma carotenoids were measured by high performance liquid chromatography as described previously [26]. Lutein, zeaxanthin, cryptoxanthin, and lycopene were detected at 476 nm, α-carotene and β-carotene at 452 nm, and retinol and retinyl palmitate at 325 nm. Total carotenoid level was calculated as the sum of retinyl palmitate, retinol, α-carotene, β-carotene, lutein, zeaxanthin, cryptoxanthin, and lycopene. For these analyses, coefficients of variance (CV) was below 2.1% for all components except retinyl palmitate (4.0%). The coefficient of variance to minimize the number of comparisons, levels of α-carotene, β-carotene, lycopene and total carotenoids were chosen to be reported before analyzing data.

Germline DNA and Genotyping

Purification of the buffy coat was carried out within 2 hr of blood draw. Each tube was centrifuged for 20 min at 1,720*g* at room temperature, the upper plasma layer was discarded

and the lymphocyte and monocyte band transferred into a 15 ml falcon tube using a sterile transfer pipette. Ten milliliter of phosphate buffered saline (PBS) was added and the tubes were centrifuged for 15 min at 300*g*. The supernatant was discarded and the cell sediment again re-suspended in 15 ml PBS and centrifuged (10 min, 300*g*). After discarding the supernatant, the remaining cell pellet was re-suspended in 1.8 ml cell preservation medium (10% DMSO, 10% fetal calf serum, 80% DMEM) and stored at −80°C until high molecular weight DNA isolation. High molecular weight genomic DNA was extracted using a QIAamp DNA blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions using ddH20 to elute DNA from the column. DNA concentration and quality were evaluated measuring the absorption ratio at 260/280 and 260/230 nm using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE) and standard agarose gel electrophoresis. The samples were diluted to 10 ng/ml for genotyping using the Sequenom Mas-sARRAY system. Tag SNPs were selected using the HapMap database to characterize variation within each gene (‘5 kilo-bases), identifying variants with a frequency of at least 5%. Among SNPs in linkage disequilibrium (R2 >0.8), we selected SNPs purported to be associated with antioxidant metabolism for analysis based on relevant function or previous literature [22,27]. Twenty SNPs from the following genes were tested for interactions with circulating carotenoids in this article: SOD1, SOD2, SOD3, XRCC1, and OGG1.

Tumor Tissue Macrodissection and DNA Extraction

A pathologist reviewed all available tissue slides and selected the one with the largest amount of the highest grade (Gleason score) for each individual case. The corresponding formalin-fixed paraffin embedded (FFPE) tissue block was then sectioned by the UCSF Tissue Core into slides for macrodissection. Ten unstained slides, each section cut at 15 μm thickness, and one 5 μm thickness hematoxylin and eosin (H&E) slide were made from each block. The pathologist outlined the H&E stained slide for areas with at least 70% tumor concentration (by number of nuclei) and using the H&E slide as a guide, the perimeter of the tumor tissue area was outlined on the unstained slides using a disposable scalpel. Tumor tissue was scraped from within the area outlined using the same scalpel and the tissue placed into a 1.5 ml Eppendorf tube for DNA extraction. A few cases with tumor areas that were too small for macrodissection had the tumor directly punched from the FFPE block by using a 1.5 mm diameter biopsy punch tool.

DNA was extracted using the Puregene DNA isolation kit (Gentra Systems) as per the manufacturer's instructions. Phenol/chloroform extraction was done after the Gentra kit's final elution step. This kit has yielded good quality DNA from FFPE material for aCGH in our laboratory[25,28].

Oligonucleotide Array Comparative Genomic Hybridization (Oligo aCGH)

Oligo aCGH was carried out using Human Genome microarrays (Agilent), consisting of 244,000 60-mer oligonucleotide DNA probes with approximately 9 kb average spatial resolution. Five hundred nanograms of tumor DNA was used for labeling. Reference male diploid DNA (Promega, Madison, WI) was used for comparison. The manufacturer's protocol was followed. Microarrays were scanned at 10 μm resolution using an Agilent-

G25005B scanner. All samples passed Agilent quality control assessment. Feature-level data was abstracted with Agilent Feature Extraction software. Copy number was expressed as the $\log_2$ ratio of tumor:control fluorescence intensity.

Copy Number Analysis

Copy number (CN) $\log_2$ intensity values were mapped to the human genome sequence hg18 freeze. Probes missing in more than 25% of patients and those missing annotation were removed and then duplicate probes were averaged. The CN values were then segmented using circular binary segmentation to translate noisy intensity measurements into regions of equal CN [29]. The sample-specific experimental variation was estimated from scaled median absolute deviation (MAD) of the difference between the observed and CBS values, after excluding the probes from Y chromosome. For each sample, the sample-specific variations were used as thresholds from the median CBS value to determine trichotomous gain/loss/no change status of each probe. Outlier probes were identified as those that were more than four sample-specific MAD away from their segment value. For each sample, a segment was declared to be gained or lost if the segment value was more than one sample MAD from the median segment value of the autosomes.

To measure the amount of genome altered, each clone was assigned a genomic distance equal to the sum of one half the distance between its center and that of its neighboring clones or to the end of a chromosome for the probes with only one neighbor. The genomic distances of clones that were gained or lost were summed and the resulting value represented the fraction of genome altered (FGA). To calculate the fraction of genome gained or lost, only the genomic distances of clones that were gained or lost, respectively, were considered.

Statistical Analysis

Antioxidant analyses—Levels of circulating carotenoids were stratified into quartiles. The association between carotenoids levels and high-grade prostate cancer was evaluated using three multivariable logistic regression models. In Model 1, we adjusted for age at diagnosis and plasma cholesterol to remove extraneous variation in circulating antioxidant levels due to cholesterol levels [30]. In model 2, we also included adjustment for smoking status (ever vs. never) and Caucasian race (yes/no). Among the 219/559 participants that had data on body mass index (BMI), we performed a third logistic regression model (Model 3) additionally adjusted for BMI. Trends were tested by modeling the median of each quartile as a continuous term.

Effect modification by germline genetic variants—We tested for evidence of effect modification between 20 SNPs in SOD1, SOD2, SOD3, XRCC1, and OGG1 and circulating carotenoids in relation to high grade prostate cancer. The SNPs for this analysis were selected a priori based on prior literature and the role of these genes in antioxidant metabolism. To evaluate effect modification, we created cross-product terms between the SNPs (using an additive model) and continuous carotenoid levels (log adjusted for normality). The cross-product terms were included in multivariate logistic regression models with the SNP of interest modeled using an additive model, log of the continuous carotenoid

of interest, age at diagnosis, circulating cholesterol, and race; a Wald test was used to determine if there was statistically significant evidence of effect modification.

Exploratory analyses of carotenoids and fraction of the genome altered—The association between Gleason sum and FGA (categorized into quartiles) in prostatectomy specimens was compared using Fisher's exact test. We then used linear regression to explore whether plasma antioxidant levels (dichotomized at the median) were associated with FGA, adjusting for circulating cholesterol, age at diagnosis, smoking status at diagnosis, and race. FGA was log-transformed for normality. The linear regression models were stratified by Gleason grade (Gleason grade ≤3 +4 vs. Gleason grade ≥4 +3) to determine whether circulating carotenoids were associated with FGA independent of Gleason. We estimated the percent change in FGA associated with above versus below the median carotenoid level using the coefficients in our linear regression model: $(e^{\beta})-1$.

Two-sided *P*-values <0.05 were considered statistically significant. All statistical analyses were performed using SAS (SAS Institute), except copy number analyses that were done using R software in the UCSF Computational Biology Core.

RESULTS

The study population was predominantly of Caucasian origin (86%). BMI decreased (*P* <0.001) and cholesterol levels increased (*P* <0.001) with increasing level of total circulating carotenoids (Table I). Circulating carotenoid levels were not associated with age at diagnosis, race, year of diagnosis, PSA at diagnosis, clinical T-stage, or smoking status at diagnosis.

Risk of High-Grade Prostate Cancer by Circulating Carotenoids

In a logistic regression model adjusted for age and circulating cholesterol (Model 1), the odds ratio of high-grade prostate cancer was 0.44 (95% CI: 0.23, 0.85; *P*-trend 0.01) comparing the highest and lowest quartiles of circulating total carotenoid levels (Table II). These estimates were relatively unchanged when adding adjustment for smoking and race (Model 2; OR 0.37 95% CI: 0.18, 0.75; *P*-trend: 0.004). In a similar fashion, the odds ratio of high-grade prostate cancer decreased with increasing levels of the individual carotenoids α-carotene (OR Q4 vs. Q1: 0.34; 95% CI: 0.18, 0.66; *P*-trend 0.003), β-carotene (OR Q4 vs. Q1: 0.31; 95% CI: 0.15, 0.63; *P*-trend 0.004) and lycopene (OR Q4 vs. Q1: 0.55; 95% CI: 0.28, 1.08; *P*-trend 0.05). In a sensitivity analysis, we added BMI as a covariate in the regression model (Model 3) in the subset of men with complete information on all covariates plus height and weight (n =214/559). This did not materially alter the point estimates for decreasing risk of high-grade prostate cancer with increasing levels of total carotenoids (OR Q4 vs. Q1: 0.45; 95% CI: 0.11, 1.80), α-carotene (OR Q4 vs. Q1: 0.51; 95% CI: 0.14, 1.79), β-carotene (OR Q4 vs. Q1: 0.52; 95% CI: 0.11, 2.51), or lycopene (OR Q4 vs. Q1: 0.51; 95% CI: 0.14, 1.94), though the associations were not statistically significant in this smaller subset of patients (data not shown).

Effect Modification by Single Nucleotide Polymorphisms

We observed an interaction between rs25489 in the XRCC1 gene, levels of circulating α-carotene and the risk of high-grade prostate cancer (*P*-interaction: 0.04), with a pronounced decreased risk of high-grade cancer among men with the GG allele in the highest quartile of α-carotene levels (OR 0.28; 95% CI: 0.14, 0.57; *P*-trend <0.001). There was no association between α-carotene and risk of high-grade prostate cancer among men with the AG genotype. In addition, men carrying the TC/CC genotype of rs699473 in the SOD3 gene had significantly lower risk of high-grade prostate cancer with increasing levels of β-carotene (*P*-interaction: 0.04; OR comparing extreme quartiles 0.20; 95% CI 0.09, 0.45; *P*-trend: <0.001). In contrast, there was no association between β-carotene and risk of high-grade prostate cancer among men with the TT genotype. Lastly, rs1052133 in the OGG1 gene modified the association between lycopene levels and high-grade cancer (*P*-interaction: 0.05). Among men carrying at least one G allele, the OR comparing extreme lycopene quartiles was 0.39 (95% CI: 0.15, 1.00; *P*-trend: 0.03); there was no association between lycopene and risk of high-grade prostate cancer among men with the CC genotype.

Overall Genomic Instability

Copy number assessment in prostate tumor tissue was available for 67 of the men who also had plasma carotenoid and cholesterol levels assessed. A frequency plot of copy number aberrations for this tumor set across the whole genome was consistent with common known genetic alterations in prostate cancer. For instance, gain of chromosomes 7q and 8q and loss of 8p, 6q and 13q, have been previously described in similar study populations [25,31]. The amount of copy number aberration across a particular tumor genome (fraction of the genome altered; FGA) was used as a metric for the overall genomic instability of a given tumor. As expected, the proportion of men with high degree of FGA increased with Gleason Score in the prostatectomy specimens as shown in Table III (*P* <0.001).

Tumor Genomic Instability and Circulating Antioxidants

In an exploratory analysis, we observed that, among men with Gleason score ≤3 +4, higher plasma lycopene levels were associated with lower genomic instability, that is, a smaller fraction of their tumor genome had copy number aberrations (*P* =0.04). The other individual carotenoids and total carotenoids were not associated with the genomic instability among men with low grade disease, and there were no statistically significant associations between plasma antioxidants and level of genome instability among men with high-grade disease (Table IV).

DISCUSSION

In this cross-sectional study, we observed that men with high circulating levels of carotenoids, including α-carotene, β-carotene and lycopene, at the time of radical prostatectomy for non-metastatic prostate cancer, had a lower risk of high-grade disease (Gleason Score ≥4 +3). Further, this association may be modified by SNPs in the XRCC1, SOD3, and OGG1 genes. In secondary exploratory analyses, we also observed that lycopene levels at the time of radical prostatectomy were inversely associated with tumor genomic instability among men with low-grade disease.

Carotenoids and Prostate Cancer

Carotenoids have been extensively studied in relation to the risk of developing prostate cancer, with inconsistent results. An early case-control study reported that lycopene, but not α-carotene or β-carotene, intake was associated with a lower risk of prostate cancer (RR 0.79; 95% CI 0.64, 0.99), sparking interest in tomato-based foods to reduce cancer incidence [2]. Later observational studies reported heterogenous results, both supporting a protective role of lycopene or tomato-based foods [3,6–8], β-carotene [5,6], and α-carotene, [6] as well as observing no protective effect [9–12]. Studies of the association between circulating levels of carotenoids in plasma and prostate cancer risk have generated similarly mixed results [14–20]. Illustrating the heterogeneous evidence, the FDA concluded that there was "very limited evidence to support an association between tomato consumption and reduced risk of prostate cancer" in 2007 [32], but the World Cancer Research Fund suggested a "likely relationship" the same year [33]. Generally, studies with cases diagnosed before the PSA-era, thus including fewer indolent cancers, have reported more robust associations.

Though data are sparse, the evidence more consistently supports a modest protective role of carotenoids on prostate cancer progression, rather than its initiation or total incidence [8,16,21]. Comparing extreme quartiles of carotenoid levels, we report that all three common carotenoids (alpha-, beta-, and lycopene) were strongly inversely associated with the risk of high-grade cancer at surgery (HR 0.31–HR 0.55), supporting the hypothesis that carotenoids may have a role in the etiology of aggressive prostate cancer or prostate cancer progression.

In addition, we report that the SNP rs25489 in XRCC1 modified the association between α-carotene and high-grade prostate cancer. The protein product of XRCC1 is involved in a number of DNA repair pathways, including base excision repair. A previous meta-analysis on XRCC1 and incident prostate cancer failed to identify any association, but recently rs915927 in XRCC1 was shown to be associated with lethal prostate cancer in men with a family history of prostate cancer [34,35]. Though exploratory, our finding supports the biological plausibility that DNA repair genes interact with dietary antioxidants to modulate cancer risk, and is in line with previous reports [36].

We also report that rs699473 in SOD3 modified the association between β-carotene and high-grade prostate cancer. SOD3 is one of three Superoxidedismutases (SODs), endogenous antioxidants that catalyse the breakdown of superoxide, thus protecting the cell from superoxide toxicity. Reduced expression of SOD3 has been reported in prostate cancer tissue and we previously reported a primary association between rs699473 and high-grade prostate cancer [26,37].

Lastly, rs1052133 in OGG1 modified the association between lycopene levels and high-grade cancer in our study population. We previously reported this SNP to similarly modify the association between selenium levels and high-grade prostate cancer [38]. While these interactions may be chance findings, the role of XRCC1, SOD3, and OGG1 in prostate cancer progression warrants further investigation, particularly in regard to antioxidants such as carotenoids.

Carotenoids, Genomic Instability, and High-Grade Prostate Cancer

We observed that the level of genomic instability, expressed as FGA, increases with Gleason Score. This result is consistent with our prior published results that FGA is positively associated with risk of recurrence after primary treatment for prostate cancer [25].

In exploratory analyses, we observed that high levels of lycopene were associated with lower genomic instability among men with low-grade tumors. There was no association between lycopene and tumor genomic stability among men with high-grade tumors, perhaps due to limited variability in FGA among men with poorly differentiated disease. In addition, the observed association was specific to lycopene; α-carotene, β-carotene, and total carotenoids were not associated with genomic instability in the prostate. While this exploratory finding may be due to chance, it is consistent with the hypothesis that lycopene inhibits the progression of prostate cancer early in its natural history. For example, Zu et al. reported that lycopene intake measured several years before diagnosis was more strongly associated with risk of lethal prostate cancer than lycopene intake at the time of diagnosis [8]. They also reported that lycopene intake was associated with the size and shape of blood vessels in prostatectomy samples. Together, the data support the hypothesis that higher lycopene levels are associated with more favorable biological characteristics in prostate tumors. This study has a number of limitations. First, our small sample size limited the statistical power, especially in analyses regarding genomic instability. Second, we lacked data on potential confounders, such as physical activity or dietary habits. However, BMI data were available for a subset of men, and point estimates were stable when adding this covariate. Third, the studied cohort was of primarily Caucasian origin. While this limits confounding due to population stratification, it also limits the external generalizability of our results to populations with different racial/ethnic distribution. Fourth, we relied on a single measurement of plasma carotenoids, and it is unknown when during the natural history of prostate cancer carotenoid levels may have the most impact. However, plasma carotenoid levels have been reported to be relatively stable over time, suggesting that one measure may reflect long-term levels [39]. We also acknowledge a risk of false positives due to multiple testing, as well as that observed associations with SNPs might be due to linkage disequilibrium with unmeasured genetic variants. Lastly, this was a cross-sectional study, and it is possible that the prostate tumor was affecting circulating carotenoid levels, rather than the carotenoid levels affecting differentiation of the tumor. Thus these results need to be replicated in future prospective studies with a larger sample size and sufficient follow-up to examine risk of recurrence and survival.

In conclusion, these data provide evidence that circulating carotenoids at diagnosis and tumor instability are associated with high-grade prostate cancer. Further studies delineating the interplay between prostate cancer aggressiveness, antioxidant status and genomic instability are warranted.

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TABLE I

Descriptive Statistics of 559 Men Initially Diagnosed With Non-Metastatic Prostate Cancer, by Quartiles of Total Circulating Carotenoids*

	Total	Q1	Q2	Q3	Q4	*P*-value
No. of men	559	138	141	139	141	
Age at diagnosis, y, mean ± SD	59.0 ± 6.9	58.6 ± 6.9	58.8 ± 6.7	60.0 ± 7.0	58.8 ± 6.8	0.5
Caucasian, N (%)	483 (86)	117 (85)	123 (87)	121 (87)	122 (87)	0.59
Year of diagnosis, median (IQR)	2004 (2002, 2006)	2005 (2002, 2006)	2005 (2003, 2006)	2004 (2002, 2006)	2004 (2003, 2006)	0.18
PSA at diagnosis, ng/ml, median (IQR)	5.8 (4.6, 8.4)	6.1 (4.8, 9.5)	5.8 (4.6, 8.2)	5.7 (4.5, 7.9)	5.8 (4.5, 7.8)	0.42
Clinical Stage, N (%)						0.86
T2b or less	142 (25)	36 (26)	34 (24)	40 (29)	32 (23)	
T2c	294 (53)	71 (51)	76 (54)	67 (48)	80 (57)	
T3a or higher	123 (22)	31 (22)	31 (22)	32 (23)	29 (21)	
Gleason score, N (%)						0.07
≤6	200 (36)	52 (38)	53 (38)	45 (32)	50 (35)	
7 (3 +4)	247 (44)	52 (38)	56 (40)	67 (48)	72 (51)	
7 (4 +3)	75 (13)	22 (16)	17 (12)	22 (16)	14 (10)	
≥8	37 (7)	12 (9)	15 (11)	5 (4)	5 (4)	
Cholesterol, mg/dl, mean ± SD	170.2 ± 37.2	150.3 ± 44.8	166.4 ± 30.7	178.6 ± 32.4	185.2 ± 29.4	<0.001
BMI at diagnosis, kg/m^2, mean ± SD[a]	27.0 ± 3.6	28.8 ± 3.6	27.5 ± 4.1	26.5 ± 2.8	25.2 ± 3.0	<0.001
Ever smoker at diagnosis, N (%)	207 (37)	52 (38)	58 (41)	52 (37)	45 (32)	0.15

*Total carotenoids is the sum of retinyl palmitate, retinol, α-carotene, β-carotene, lutein, zeazanthin, cryptoxanthin, and lycopene.

[a]Body mass index (BMI) was available on 219 participants: 49 men in Q1; 58 men in Q2; 56 men in Q3; and 56 men in Q4.

TABLE II

Relative Risk of High-Grade Prostate Cancer Among 559 Men Initially Diagnosed With Non-Metastatic Prostate Cancer, by Circulating Carotenoids*

	Quartile of carotenoid				
	Q1	**Q2**	**Q3**	**Q4**	***P*-value[a]**
Total carotenoids[b]					
Number of events	34	32	27	19	
Model 1[c]	1.0 (ref.)	0.86 (0.49, 1.51)	0.67 (0.37, 1.21)	0.44 (0.23, 0.85)	0.01
Model 2[d]	1.0 (ref.)	0.77 (0.43, 1.39)	0.56 (0.30, 1.06)	0.37 (0.18, 0.75)	0.004
α-carotene					
Number of events	39	23	31	19	
Model 1[c]	1.0 (ref.)	0.47 (0.26, 0.85)	0.69 (0.40, 1.20)	0.37 (0.20, 0.68)	0.006
Model 2[d]	1.0 (ref.)	0.46 (0.25, 0.86)	0.59 (0.32, 1.07)	0.34 (0.18, 0.66)	0.003
β-carotene					
Number of events	36	25	34	17	
Model 1[c]	1.0 (ref.)	0.60 (0.34, 1.08)	0.88 (0.51, 1.52)	0.36 (0.19, 0.69)	0.01
Model 2[d]	1.0 (ref.)	0.57 (0.31, 1.06)	0.76 (0.43, 1.37)	0.31 (0.15, 0.63)	0.004
Lycopene					
Number of events	33	33	26	20	
Model 1[c]	1.0 (ref.)	0.95 (0.55, 1.67)	0.70 (0.39, 1.27)	0.52 (0.27, 1.00)	0.03
Model 2[d]	1.0 (ref.)	0.94 (0.51, 1.70)	0.62 (0.33, 1.18)	0.55 (0.28, 1.08)	0.05

*Total Gleason ≥8 or Gleason =7 with primary score ≥4.

[a]P-trend calculated by modeling the median of each quartile as a continuous term.

[b]Total carotenoids include the sum of retinyl palmitate, retinol, α-carotene, β-carotene, lutein, zeazanthin, cryptoxanthin, and lycopene.

[c]Multivariate logistic regression model adjusted for age at diagnosis (years) and circulating cholesterol (mg/dL).

[d]Multivariate logistic regression model adjusted for variables in Model 1 plus smoking status at diagnosis (ever vs. never) and Caucasian race (yes/no).

TABLE III

Gleason Sum and Genomic Instability in Prostate Tumor Tissue Among 67 Men With Non-Metastatic Prostate Cancer Who Underwent radical prostatectomy at the University of California, San Francisco

	Quartile of fraction of the genome altered[a]				
Pathologic gleason sum	**Q1**	**Q2**	**Q3**	**Q4**	**Fisher's exact *P*-value**
2–6	13	12	5	1	<0.001
7	3	1	4	2	
8–10	0	4	8	14	

[a]Sixty-seven out of the 559 men had data available on fraction of the genome altered, a measure of genomic instability in the prostate tumor.

TABLE IV

Linear Regression* the Relation Between Plasma Antioxidants and Genomic Instability in Prostate Tumor Tissue Among 60 Men With Non-Metastatic Prostate Cancer Who Underwent Radical Prostatectomy at the University of California, San Francisco, Stratified by Pathologic Gleason Sum

	Gleason sum ≤3 +4 (n=32)			Gleason sum ≥4 +3 (n =28)			Total (n =60)		
Plasma carotenoids	**β ± SE***	**% Δ in FGA*a***	***P*-value**	**β ± SE**	**% Δ in FGA*b***	***P*-value**	**β ± SE**	**% Δ in FGA*b***	***P*-value**
Total carotenoids*b*	−0.15 ± 0.46	−14	0.74	−0.23 ± 0.35	−21	0.52	−0.21 ± 0.28	−19	0.45
α-carotene	0.71 ± 0.42	103	0.11	−0.20 ± 0.33	−18	0.55	0.27 ± 0.27	31	0.32
β-carotene	0.25 ± 0.42	28	0.55	−0.00 ± 0.31	0	0.99	0.16 ± 0.27	17	0.55
Lycopene	−0.92 ± 0.44	−60	0.04	0.40 ± 0.31	49	0.2	−0.22 ± 0.28	−20	0.42

*Linear regression models examining high versus low plasma carotenoids (dichotomized at the median) in relation to fraction of the genome altered (FGA), adjusted for age at diagnosis (year), circulating cholesterol (mg/dl), race (white vs. not white), and smoking status at diagnosis (current vs. never). Seven of the 67 men with circulating carotenoids and FGA data were missing data on smoking status at diagnosis and were not included.

*a*Percent change in FGA comparing high versus low plasma antioxidants (dichotomized at the median) was calculated as follows: $[(e^{\beta}) - 1] \times 100$.

*b*Total carotenoids is the sum of retinyl palmitate, retinol, α-carotene, β-carotene, lutein, zeazanthin, cryptoxanthin, and lycopene.