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Original Contribution

Serum Phospholipid Fatty Acids, Genetic Variation in Myeloperoxidase, and Prostate Cancer Risk in Heavy Smokers: A Gene-Nutrient Interaction in the Carotene and Retinol Efficacy Trial

Ting-Yuan David Cheng*, Irena B. King, Matt J. Barnett, Christine B. Ambrosone, Mark D. Thornquist, Gary E. Goodman, and Marian L. Neuhouser

* Correspondence to Ting-Yuan David Cheng, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M4B402, Seattle, WA 98109-1024 (e-mail: tcheng@fhcrc.org).

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The authors investigated associations of serum phospholipid *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs) and *trans*-fatty acids with prostate cancer risk, and whether myeloperoxidase G-463A (rs2333227) modified the associations in the Carotene and Retinol Efficacy Trial (CARET) (Seattle, Washington; Irvine, California; New Haven, Connecticut; San Francisco, California; Baltimore, Maryland; and Portland, Oregon, 1985–2003). Prerandomization sera were assayed for fatty acids among 641 men with incident prostate cancer (368 nonaggressive and 273 aggressive (stage III/IV or Gleason score ≥7)) and 1,398 controls. Overall, dihomo-γ-linolenic (quartiles 4 vs. 1: odds ratio (OR) = 0.66, 95% confidence interval (CI): 0.49, 0.95; $P_{trend} = 0.024$) and docosatetraenoic (OR = 0.69, 95% CI: 0.46, 1.02; $P_{trend} = 0.011$) acids were inversely associated with nonaggressive and aggressive prostate cancer risks, respectively. Among men with *MPO* GG, the genotype upregulating oxidative stress, quartiles 4 versus 1 eicosapentaenoic plus docosahexaenoic acids were suggestively associated with an increased risk of aggressive prostate cancer (OR = 1.66, 95% CI: 0.95, 2.92; $P_{trend} = 0.07$). However, the association was the inverse among men with *MPO* GA/AA genotypes ($P_{interaction} = 0.011$). Interactions were also observed for docosapentaenoic acid, total *n*-3 PUFAs, and arachidonic acid. *MPO* GA/AA vs. GG was associated with a 2-fold increase in aggressive prostate cancer risk among men with low (quartile 1) *n*-3 PUFAs. This study adds important evidence linking oxidative stress with prostate carcinogenesis.

gene-environment interaction; myeloperoxidase; polyunsaturated fatty acids; prostate cancer; *trans*-fatty acids

Abbreviations: CARET, Carotene and Retinol Efficacy Trial; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PUFA, polyunsaturated fatty acid.

Biological evidence supports a role for phospholipid fatty acid in prostate carcinogenesis (1–3). Among major types of fatty acids, polyunsaturated fatty acids (PUFAs), including the *n*-6 and *n*-3 PUFAs, are essential to cell membranes and inflammation signaling (4). *n*-6 PUFAs promote androgen-stimulated prostate cell growth, but long-chain and very-long-chain *n*-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), inhibit this pathway (2). *n*-3 PUFAs also have antiinflammatory, antiproliferative, and proapoptotic effects on prostate cancer cells (2). However, the multiple double bonds present in both *n*-6 and *n*-3 PUFAs attract reactive oxygen species or free radicals. This process is known as lipid peroxidation; that is, free radicals take electrons from the lipids in cell membranes, leading to membrane and DNA damage, which favors cancer development, including prostate cancer (5, 6).

An important determinant of lipid peroxidation is oxidative-stress regulatory enzymes, which metabolize free radicals and thus protect PUFAs from peroxidation (7, 8). Among the family of oxidative-stress regulatory enzymes, myeloperoxidase

converts hydrogen peroxide (H_2O_2), the metabolite generated from superoxide dismutases, and chloride anion (Cl^-) into hypochlorous acid (HOCl), a secondary, endogenous free radical peroxidizing PUFAs (9). Previous reports from the Carotene and Retinol Efficacy Trial (CARET) have linked *MPO* G-463A (rs2333227), a functional single nucleotide polymorphism of myeloperoxidase, to prostate cancer risk. The *MPO* A allele, conferring several folds less transcriptional activity than the G allele (10), is associated with a 60% reduced risk of aggressive prostate cancer (11). Also, *MPO* G-463A modifies the association of serum α-tocopherol, the body's primary fat-soluble antioxidant, with aggressive prostate cancer among CARET current smokers (12). Both *n*-3 and *n*-6 PUFAs are potentially prooxidative because of their double bonds, but their interaction with oxidative-stress regulatory enzymes has not been investigated in epidemiologic studies. The primary objective of this study was to examine the associations of serum phospholipid *n*-3 and *n*-6 PUFAs as well as *trans*-fatty acids and prostate cancer risk in CARET. We included *trans*-fatty acids because an increased prostate cancer risk was observed with higher levels of C18 *trans*-fatty acids in CARET (13), and the biological mechanisms may involve oxidative stress (14). We further investigated whether the *MPO* G-463A polymorphism modified the associations. We hypothesized that high percentages of PUFAs in the presence of the genotype producing high oxidative stress (GG) were associated with an increased risk of prostate cancer but not in the presence of the genotype producing low oxidative stress (GA/AA).

MATERIALS AND METHODS

CARET overview

CARET was a multicenter (Seattle, Washington; Irvine, California; New Haven, Connecticut; San Francisco, California; Baltimore, Maryland; and Portland, Oregon) randomized, double-blind placebo-controlled chemoprevention trial to test whether daily supplementation with 30 mg of β-carotene and 25,000 IU of retinyl palmitate would reduce the risk of lung cancer among 18,314 heavy smokers and asbestos-exposed workers. Details about the design and primary results of CARET have been published elsewhere (15). Briefly, eligible participants were men and women aged 50–69 years who were current or former smokers (within the previous 6 years) with a history of at least 20 pack-years of cigarette smoking (n = 14,254, 55.9% of whom were male) and men aged 45–69 years who were current or former smokers and exposed to asbestos in the workplace within the previous 15 years (n = 4,060). Recruitment began in 1985 and intervention was stopped in 1996; 94% of participants remained in active follow-up until 2005. The institutional review boards of the Fred Hutchinson Cancer Research Center and each of the 5 other participating institutions approved all procedures for the study, and participants provided written informed consent at recruitment and throughout the trial. For the analyses in this study, additional institutional review was obtained from the Roswell Park Cancer Institute.

Selection of cases and controls and endpoint ascertainment

We used a nested case-control study. At each CARET annual visit, as well as quarterly follow-up telephone calls, participants were asked to report if they had been diagnosed with any new cancers. All endpoints, including prostate cancer, were verified by the CARET Endpoints Review Committee. For the current study, participants with a previous report of prostate cancer were approached to request permission to obtain data on Gleason score and stage of disease at diagnosis through review of their medical records. For participants whose medical records were unavailable, the stage and Gleason score were obtained from pathology reports in the Western Washington Cancer Surveillance System, a part of the Surveillance, Epidemiology, and End Results registries. All medical and pathologic records were confirmed by one of the coauthors (G.E.G.). Case selection for this study was based on follow-up through 2003, by which time a total of 778 incident prostate cancer cases had been confirmed. After exclusion of 50 men with prior cancer history reported at the baseline visit and 19 without specimens available for laboratory analyses, 709 cases were eligible for this study (16). Eligible controls were men who were free of both prostate cancer and lung cancer at the time of selection (follow-up through 2003) and had available whole blood or extracted DNA. Biospecimens of lung cancer cases (the primary endpoint in CARET) were not provided for studies not investigating lung cancer. Cases and controls were frequency matched on age (5-year groups) and race/ethnicity, and controls were required to have follow-up time at least that of their matched case. The case:control ratios were 1:4 for blacks, wherever achievable, and 1:2 for other races. As a result, a total of 724 cases and 1,474 controls were selected (after reassigning 15 participants who were originally selected as controls and diagnosed subsequently with prostate cancer). Forty-five cases and 44 controls did not have data on serum phospholipid fatty acids because of insufficient specimens. In addition, 14 cases and 32 controls did not have complete baseline data on covariates. Staging information and Gleason scores were available for 87% and 93% of the cases, respectively (16). Consequently, 641 cases with known staging or Gleason score and 1,398 controls entered statistical analyses for the main associations of PUFAs and *trans*-fatty acids with prostate cancer. After exclusion of those without complete genotyping data, the analysis of interaction between genetic variation in *MPO* and those fatty acids was conducted in 458 cases and 1,369 controls. The missing genotyping data in the cases were mainly because whole blood collection was not initiated until 1994. For the whole blood collection, the overall rates of consent and completion were 70%.

Serum phospholipid fatty acid assay and *MPO genotyping*

Participants provided nonfasting blood specimens at their first CARET study center visit (prerandomization). Sera were stored in the CARET Coordinating Center specimen bank at −70°C until analysis. Total lipids were extracted by

the method of Folch et al. (17), and phospholipids were separated from neutral lipids by one-dimensional thin-layer chromatography using 250-μm silica gel G plates and a 67.5:15:0.75 hexane:ether:acetic acid (0.005% butylated hydroxytoluene) development solvent. Samples of fatty acid methyl esters were prepared by direct transesterification using the method of Lepage and Roy (18). A gas chromatograph (model 5890B, series II; Hewlett-Packard, Avondale, Pennsylvania) equipped with a flame ionization detector, an automatic sampler (model 7673; Hewlett-Packard), and electronic pressure programming was used on samples dissolved in hexane. Fatty acid methyl esters were separated on a SP-2560 wall-coated open-tubular fused silica capillary column, 100 m × 0.25 mm inner-diameter, 0.20-μm film thickness (Supelco, Bellefonte, Pennsylvania). The carrier gas was helium. This method yielded 41 individual phospholipid fatty acids in total. Quantitative precision and identification were evaluated by using model mixtures of known fatty acid methyl esters and an established control pool. Interassay coefficients of variation were on the average 3.5% or lower for most of the fatty acids that were present at levels of 1% or higher. Individual fatty acids were expressed as a weight percentage of the total fatty acids.

The polymorphism in *MPO* G-463A (rs2333227) was selected for genotyping because this variant substantially influences the capacity for responding to oxidative stress (10). Genomic DNA was extracted with the use of QIAamp DNA blood Midi kits (Qiagen, Valencia, California). Genotyping was performed with high-throughput matrix-assisted laser desorption/ionizing time-of-flight mass spectrometry (Sequenom, San Diego, California) by BioServe Biotechnologies (Laurel, Maryland). Procedures and primers for polymerase chain reaction were previously reported (19). The genotype concordance was excellent among the 8% of randomly selected duplicates (k statistic: 0.95) with a <1% assay failure rate. The polymorphism was in Hardy-Weinberg equilibrium among the controls; thus, selection bias or genotyping error was unlikely.

Other data collection

Detailed information on demographic characteristics, smoking status, and personal and family health history was collected by a self-administered questionnaire at baseline. Current smokers were defined as those who smoked any cigarettes in the past month. The age of starting smoking and quitting smoking (for former smokers) and the average number of cigarettes smoked per day for the entire time of smoking were also assessed to calculate smoking pack-years. Height and weight were measured by trained staff using a standardized protocol at the baseline visit, and body mass index (weight (kg)/height $(m)^2$) was calculated. Alcohol consumption was estimated from the CARET food frequency questionnaire, which was administered at baseline and then every 2 years during the trial. Alcohol intake values were averaged across all food frequency questionnaires completed prior to the prostate cancer diagnosis to best represent the long-term pattern of alcohol intake during follow-up.

Statistical analyses

Our analytical goals were to assess the association of individual fatty acids (main effect) and the joint association of individual fatty acids and the *MPO* G-463A genetic variation (effect modification) with prostate cancer risk. The percentages of individual *n*-3 and *n*-6 PUFAs as well as total *trans* C16 monounsaturated, C18 monounsaturated, and C18:2 diunsaturated fatty acids were categorized into quartiles on the basis of their distributions in the controls. Unconditional logistic regression was used to estimate odds ratios and 95% confidence intervals. Tests for linear trend across the quartiles were based on an ordinal variable corresponding to rank from the first to fourth quartiles. A covariate was included in multivariate models if a priori knowledge suggested that the variable was a confounder. The multivariate models included age at enrollment (continuous), race (white, black, or others), CARET randomization assignment (retinol plus β-carotene or placebo), family history of prostate cancer in first-degree relatives (yes or no), alcohol consumption (nondrinker, below median, or at or above median based on total alcohol amount in controls reporting use of alcohol where the median intake was 10 g/day), smoking status (current or former/never), smoking pack-years (<40, 40–59, or ≥60), and body mass index (continuous). Analyses were conducted for nonaggressive and aggressive prostate cancer separately, where aggressive prostate cancer was defined as clinical stage III or IV (extraprostatic extension or metastasis) tumors or with Gleason score ≥7 (11, 20). A secondary analysis was conducted to assess risks of 1) advanced stage prostate cancer, defined as stage III or IV (either clinical or pathological); 2) high-grade prostate cancer, defined as Gleason score ≥8; and 3) lethal prostate cancer, defined as metastatic tumor (clinical or pathological stage IV at diagnosis) or prostate cancer–specific death during follow-up through 2005 (21). To explore effect modification, the reference group for a given model was men in the lowest quartile of serum fatty acid percentages and with the *MPO* GG genotype. Participants with heterozygote alleles and homozygote A alleles were combined into 1 group since the 2 genotypes have the same transcriptional activity (10, 22). A cross-product term of the ordinal variable of fatty acid quartiles and the *MPO* genotypes was created; the interaction was based on likelihood ratio tests (1 df). All tests were 2-sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed by using Stata, version 12, software (StataCorp LP, College Station, Texas).

RESULTS

Table 1 gives the characteristics of prostate cancer cases and controls in CARET. Compared with controls, higher proportions of cases had a family history of prostate cancer ($P < 0.001$) and high alcohol consumption ($P = 0.010$). Table 2 presents the 25th, 50th (or median), and 75th percentiles of serum *n*-3 and *n*-6 PUFAs and *trans*-fatty acids as the percentage of total phospholipid fatty acids in nonaggressive and aggressive prostate cancer cases and controls. Among controls, approximately 4% and 35% of total fatty

Table 1. Characteristics of Prostate Cancer Cases and Controls in the Carotene and Retinol Efficacy Trial, 1985–2003

Characteristics	Cases			Controls			*P* Value[a]
	Mean (SD)	No.[b]	%	Mean (SD)	No.[b]	%	
Total		641			1,398		
Age, years							
Baseline	60.4 (5.7)			60.3 (5.8)			0.56
Diagnosis	66.9 (5.9)			N/A			
Race/ethnicity							
White		578	90.2		1,229	87.9	0.13
African American		39	6.1		121	8.7	
Other		24	3.7		48	3.4	
Randomization							
Intervention		334	52.1		724	51.8	0.89
Placebo		307	47.9		674	48.2	
Family history of prostate cancer, yes		42	6.6		46	3.3	<0.001
Smoking status							
Current		332	51.8		741	53.0	0.61
Never[c]/former		309	48.2		657	47.0	
Smoking, pack-years							
<40		238	37.1		530	37.9	0.85
40–59		216	33.7		477	34.1	
≥60		187	29.2		391	28.0	
Alcohol intake							
Nondrinkers		145	22.6		341	24.4	0.010
1–9 g/day		181	28.2		475	34.0	
≥10 g/day		262	40.9		472	33.8	
Unknown		53	8.3		110	7.9	
Body mass index[d]							
<25.0		147	22.9		305	21.8	0.84
25.0–29.9		309	48.2		689	49.3	
≥30.0		185	28.9		404	28.9	
Gleason score							
<7		361	56.3		N/A		
≥7		258	40.3				
Unknown		22	3.4				
Clinical stage							
0, I		168	33.5		N/A		
II		280	55.8				
III		26	5.2				
IV		25	5.0				
Unknown		3	0.6				
Year of diagnosis[e]					N/A		
1986–1993		145	22.6				
1994–2003		496	77.4				

Abbreviations: CARET, Carotene and Retinol Efficacy Trial; N/A, nonapplicable; PSA, prostate-specific antigen; SD, standard deviation.

[a] The *t*-test for age at baseline and χ^2 tests for the categorical variables were used.

[b] The numbers are numbers of participants and column percentages unless otherwise noted.

[c] Never smokers contributed a very small percentage (<2%). They were recruited in the CARET because of their occupational asbestos exposure.

[d] Body mass index: weight (kg)/height $(m)^2$.

[e] 1994 approximates the advent of the PSA era.

Table 2. Distributions of Serum Fatty Acid Composition as Total Phospholipids (%)[a] Shown as the 25th, 50th (Median), and 75th Percentiles in the Carotene and Retinol Efficacy Trial, 1985–2003

Fatty Acids	Nonaggressive Prostate Cancer Cases, %[b]			Aggressive Prostate Cancer Cases, %[c]			Controls, %[d]		
	25th Percentile	Median	75th Percentile	25th Percentile	Median	75th Percentile	25th Percentile	Median	75th Percentile
n-3 PUFAs									
18:3*n*-3 (α-linolenic)	0.09	0.10	0.11	0.09	0.10	0.11	0.09	0.10	0.12
20:3*n*-3 (eicosatrienoic)	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02
20:5*n*-3 (eicosapentaenoic)	0.45	0.58	0.75	0.44	0.59	0.76	0.42	0.56	0.75
22:5*n*-3 (docosapentaenoic)	0.71	0.80	0.90	0.71	0.81	0.91	0.71	0.81	0.91
22:6*n*-3 (docosahexaenoic)	2.11	2.59	3.14	2.12	2.52	3.14	2.09	2.56	3.16
Total *n*-3	3.52	4.08	4.72	3.53	4.08	4.81	3.53	4.05	4.76
n-6 PUFAs									
18:2*n*-6 (linoleic)	18.76	20.55	22.29	18.95	20.75	22.44	18.83	20.57	22.32
18:3*n*-6 (γ-linolenic)	0.05	0.07	0.09	0.05	0.07	0.09	0.05	0.07	0.09
20:2*n*-6 (eicosadienoic)	0.30	0.33	0.37	0.31	0.34	0.38	0.31	0.34	0.38
20:3*n*-6 (dihomo-γ-linolenic)	2.51	2.84	3.22	2.52	2.95	3.45	2.54	2.94	3.40
20:4*n*-6 (arachidonic)	9.44	10.77	12.17	9.31	10.47	11.52	9.52	10.64	11.82
22:2*n*-6 (docosadienoic)	0.02	0.03	0.04	0.03	0.03	0.04	0.02	0.03	0.04
22:4*n*-6 (docosatetraenoic)	0.39	0.45	0.51	0.39	0.44	0.50	0.39	0.46	0.52
Total *n*-6	34.11	35.10	36.58	33.82	35.33	36.39	34.07	35.26	36.41
TFA 16:1	0.17	0.20	0.24	0.16	0.20	0.24	0.17	0.20	0.24
TFA 18:1	1.20	1.63	2.07	1.18	1.59	2.12	1.20	1.59	2.11
TFA 18:2	0.18	0.23	0.28	0.18	0.23	0.28	0.18	0.22	0.28

Abbreviations: PUFA, polyunsaturated fatty acid; TFA, *trans*-fatty acid.

[a] The summation of fatty acid shown in this table is not 100% because other groups (saturated and monounsaturated) of fatty acids are not listed.

[b] There were 368 cases with nonaggressive prostate cancer defined as stage 0–II tumors and Gleason score <7.

[c] There were 273 cases with aggressive prostate cancer defined as stage III/IV tumors or Gleason score ≥7.

[d] There were 1,398 controls.

acids were composed of *n*-3 and *n*-6 PUFAs, respectively. The largest components were linoleic acid (20.57%) followed by arachidonic acid (10.64%) among the *n*-6 PUFAs and DHA (2.56%) among the *n*-3 PUFAs.

In the main effect analysis, no significant association was observed for *n*-3 PUFAs (Tables 3 and 4) or for *trans*-fatty acids (Web Table 1 available at http://aje.oxfordjournals.org/), but 2 *n*-6 PUFAs were inversely associated with prostate cancer risk. Men with dihomo-γ-linolenic acid percentages in the fourth quartile were at 34% lower risk for nonaggressive prostate cancer, compared with those with the percentages in the first quartile (odds ratio (OR) = 0.66, 95% confidence interval (CI): 0.47, 0.95; $P_{trend} = 0.024$) (Table 3). Docosatetraenoic acid was inversely associated with aggressive prostate cancer risk (for quartiles 4 vs. 1: OR = 0.69, 95% CI: 0.46, 1.02; $P_{trend} = 0.011$) (Table 4).

No effect modification of genetic variation in *MPO* G-463A on nonaggressive prostate cancer risk was observed for *n*-3 and *n*-6 PUFAs (Web Table 2) or on any prostate cancer risk for *trans*-fatty acids (Web Table 3). However, the polymorphism significantly modified the associations of several long-chain and very-long-chain *n*-3 and *n*-6 PUFAs with aggressive prostate cancer risk (Table 5). For *n*-3 PUFAs, the *MPO* GA/AA versus GG genotypes were associated with a nearly 2-fold increase in aggressive prostate cancer risk among men with low (quartile 1) EPA + DHA (OR = 1.97, 95% CI: 1.07, 3.63). Among men with the *MPO* GG genotypes, a positive, yet nonsignificant, association

Table 3. Multivariable-adjusted[a] Association of Serum *n*-3 and *n*-6 Polyunsaturated Fatty Acids With Nonaggressive Prostate Cancer[b] Risk in the Carotene and Retinol Efficacy Trial, 1985–2003

Fatty Acids	Quartile 1				Quartile 2				Quartile 3				Quartile 4				P_{trend}
	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	
n-3 PUFAs																	
α-Linolenic acid	84	350	1.00	Referent	114	349	1.36	0.99, 1.88	88	349	1.06	0.75, 1.48	82	350	0.99	0.70, 1.41	0.59
Eicosatrienoic acid	85	350	1.00	Referent	88	349	1.07	0.77, 1.50	98	350	1.18	0.84, 1.64	96	349	1.15	0.83, 1.61	0.34
Eicosapentaenoic acid	77	350	1.00	Referent	97	349	1.24	0.89, 1.74	104	350	1.28	0.92, 1.79	90	349	1.07	0.75, 1.52	0.70
Docosapentaenoic acid	96	349	1.00	Referent	95	350	0.97	0.70, 1.33	88	349	0.88	0.63, 1.22	89	350	0.89	0.64, 1.24	0.41
Docosahexaenoic acid	88	349	1.00	Referent	93	350	1.05	0.75, 1.46	99	349	1.13	0.81, 1.58	88	350	1.00	0.70, 1.41	0.90
EPA + DHA	78	350	1.00	Referent	98	349	1.26	0.90, 1.77	102	350	1.31	0.93, 1.83	90	349	1.14	0.80, 1.63	0.45
Total *n*-3	93	350	1.00	Referent	84	349	0.90	0.64, 1.25	103	349	1.09	0.79, 1.51	88	350	0.92	0.66, 1.30	0.95
n-6 PUFAs																	
Linoleic acid	99	349	1.00	Referent	88	350	0.89	0.64, 1.23	90	350	0.91	0.65, 1.26	91	349	0.93	0.67, 1.30	0.72
γ-Linolenic acid	92	350	1.00	Referent	90	349	0.93	0.67, 1.29	91	349	0.89	0.64, 1.25	95	349	0.96	0.69, 1.34	0.78
Eicosadienoic acid	110	350	1.00	Referent	87	349	0.79	0.57, 1.09	80	350	0.71	0.51, 0.99	91	349	0.83	0.60, 1.14	0.18
Dihomo-γ-linolenic acid	101	349	1.00	Referent	104	350	1.01	0.74, 1.38	94	349	0.92	0.66, 1.27	69	350	0.66	0.47, 0.95	0.024
Arachidonic acid	99	350	1.00	Referent	76	349	0.78	0.56, 1.09	85	349	0.89	0.64, 1.24	108	350	1.16	0.84, 1.60	0.32
Docosadienoic acid	87	350	1.00	Referent	114	349	1.33	0.97, 1.83	79	349	0.92	0.66, 1.30	88	350	1.04	0.74, 1.46	0.61
Docosatetraenoic acid	95	350	1.00	Referent	98	349	1.03	0.74, 1.42	92	349	0.97	0.70, 1.34	83	350	0.89	0.63, 1.24	0.44
Total *n*-6	89	350	1.00	Referent	105	349	1.21	0.88, 1.68	72	349	0.84	0.59, 1.19	102	350	1.21	0.87, 1.68	0.66

Abbreviations: CARET, Carotene and Retinol Efficacy Trial; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PUFA, polyunsaturated fatty acid.

[a] Multivariate adjustment for age at enrollment (continuous), race (white, black, others), CARET randomization assignment (retinol plus β-carotene, placebo), family history of prostate cancer in first-degree relatives (yes, no), alcohol consumption (nondrinker, below median, at or above median, unknown), smoking status (current, former/never), smoking pack-years (<40, 40, <60, ≥60), and body mass index (continuous).

[b] Defined as stage 0–II tumors and Gleason score <7.

Table 4. Multivariable-adjusted[a] Association of Serum *n*-3 and *n*-6 Polyunsaturated Fatty Acids With Aggressive Prostate Cancer[b] Risk in the Carotene and Retinol Efficacy Trial, 1985–2003

Fatty Acids	Quartile 1				Quartile 2				Quartile 3				Quartile 4				P_{trend}
	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	
n-3 PUFAs																	
α-Linolenic acid	65	350	1.00	Referent	87	349	1.33	0.93, 1.90	62	349	0.99	0.67, 1.45	59	350	0.93	0.62, 1.37	0.39
Eicosatrienoic acid	62	350	1.00	Referent	72	349	1.20	0.82, 1.74	65	350	1.07	0.73, 1.57	74	349	1.27	0.88, 1.85	0.31
Eicosapentaenoic acid	54	350	1.00	Referent	69	349	1.25	0.85, 1.85	77	350	1.32	0.90, 1.94	73	349	1.20	0.81, 1.79	0.38
Docosapentaenoic acid	71	349	1.00	Referent	69	350	0.94	0.65, 1.36	67	349	0.93	0.64, 1.35	66	350	0.91	0.63, 1.32	0.63
Docosahexaenoic acid	63	349	1.00	Referent	81	350	1.30	0.90, 1.87	62	349	1.05	0.71, 1.56	67	350	1.10	0.74, 1.63	0.92
EPA + DHA	65	350	1.00	Referent	62	349	0.97	0.66, 1.42	79	350	1.23	0.85, 1.78	67	349	1.05	0.71, 1.55	0.53
Total *n*-3	66	350	1.00		69	349	1.07	0.74, 1.55	68	349	1.06	0.73, 1.55	70	350	1.07	0.73, 1.57	0.75
n-6 PUFAs																	
Linoleic acid	65	349	1.00	Referent	64	350	0.96	0.65, 1.40	71	350	1.07	0.73, 1.55	73	349	1.15	0.79, 1.70	0.38
γ-Linolenic acid	75	350	1.00	Referent	58	349	0.73	0.50, 1.07	71	349	0.85	0.59, 1.23	69	349	0.83	0.57, 1.20	0.48
Eicosadienoic acid	69	350	1.00	Referent	56	349	0.80	0.54, 1.18	81	350	1.19	0.83, 1.70	67	349	0.96	0.66, 1.40	0.63
Dihomo-γ-linolenic acid	70	349	1.00	Referent	65	350	0.92	0.63, 1.34	65	349	0.95	0.65, 1.38	73	350	1.03	0.71, 1.51	0.84
Arachidonic acid	73	350	1.00	Referent	72	349	1.02	0.71, 1.46	71	349	1.02	0.71, 1.46	57	350	0.83	0.56, 1.23	0.42
Docosadienoic acid	54	350	1.00	Referent	72	349	1.38	0.94, 2.03	73	349	1.39	0.94, 2.04	74	350	1.46	0.99, 2.14	0.08
Docosatetraenoic acid	76	350	1.00	Referent	91	349	1.22	0.86, 1.72	55	349	0.75	0.51, 1.10	51	350	0.69	0.46, 1.02	0.011
Total *n*-6	77	350	1.00	Referent	52	349	0.69	0.47, 1.02	77	349	1.07	0.75, 1.53	67	350	0.95	0.66, 1.38	0.70

Abbreviations: CARET, Carotene and Retinol Efficacy Trial; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PUFA, polyunsaturated fatty acid.

[a] Multivariate adjustment for age at enrollment (continuous), race (white, black, others), CARET randomization assignment (retinol plus β-carotene, placebo), family history of prostate cancer in first-degree relatives (yes, no), alcohol consumption (nondrinker, below median, at or above median, unknown), smoking status (current, former/never), smoking pack-years (<40, 40, <60, ≥60), and body mass index (continuous).

[b] Defined as stage III/IV tumors or Gleason score ≥7.

Table 5. Joint Association[a] of Serum *n*-3 and *n*-6 Polyunsaturated Fatty Acids and the *MPO* G-463A Polymorphism With Aggressive Prostate Cancer[b] in the Carotene and Retinol Efficacy Trial, 1985–2003

Fatty Acids	*MPO*[c] Genotype	Quartile 1				Quartile 2				Quartile 3				Quartile 4				P_{trend}	$P_{interaction}$
		No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI		
n-3 PUFAs																			
α-Linolenic acid	GG	28	204	1.00	Referent	39	210	1.31	0.77, 2.22	27	189	1.08	0.61, 1.91	34	217	1.16	0.67, 2.01	0.96	0.54
	GA/AA	19	138	1.01	0.54, 1.89	25	132	1.45	0.80, 2.61	19	156	0.92	0.49, 1.71	15	123	0.95	0.48, 1.87	0.80	
Eicosatrienoic acid	GG	29	202	1.00	Referent	27	209	0.92	0.52, 1.62	33	197	1.18	0.69, 2.05	39	212	1.39	0.82, 2.37	0.16	0.88
	GA/AA	14	144	0.68	0.35, 1.35	23	130	1.36	0.75, 2.48	23	145	1.18	0.65, 2.14	18	130	1.06	0.56, 2.01	0.21	
Eicosapentaenoic acid	GG	25	216	1.00	Referent	31	203	1.25	0.71, 2.20	37	197	1.47	0.84, 2.55	35	204	1.25	0.71, 2.20	0.27	0.68
	GA/AA	14	127	0.96	0.48, 1.93	23	144	1.39	0.75, 2.55	22	146	1.19	0.64, 2.22	19	132	1.11	0.58, 2.14	0.95	
Docosapentaenoic acid	GG	31	227	1.00	Referent	26	191	0.96	0.55, 1.69	38	206	1.34	0.80, 2.25	33	196	1.21	0.71, 2.06	0.24	0.013
	GA/AA	23	118	1.49	0.82, 2.70	26	150	1.28	0.73, 2.26	15	137	0.83	0.43, 1.60	14	144	0.72	0.37, 1.41	0.026	
Docosahexaenoic acid	GG	25	227	1.00	Referent	40	196	1.88	1.09, 3.24	29	206	1.37	0.77, 2.45	34	191	1.64	0.93, 2.90	0.33	0.028
	GA/AA	23	116	1.86	1.00, 3.44	24	146	1.55	0.84, 2.84	15	139	1.05	0.53, 2.08	16	148	1.09	0.55, 2.15	0.17	
EPA + DHA	GG	25	230	1.00	Referent	30	199	1.39	0.79, 2.47	38	200	1.74	1.01, 3.02	35	191	1.66	0.95, 2.92	0.07	0.011
	GA/AA	24	115	1.97	1.07, 3.63	19	144	1.24	0.65, 2.34	19	143	1.25	0.66, 2.38	16	147	1.06	0.54, 2.10	0.15	
Total *n*-3	GG	25	229	1.00	Referent	32	202	1.46	0.83, 2.56	35	201	1.64	0.94, 2.87	36	188	1.70	0.97, 2.98	0.07	0.002
	GA/AA	28	118	2.21	1.23, 4.00	19	138	1.32	0.70, 2.51	14	142	0.92	0.46, 1.84	17	151	1.10	0.56, 2.14	0.033	
n-6 PUFAs																			
Linoleic acid	GG	25	202	1.00	Referent	39	193	1.61	0.93, 2.78	32	197	1.31	0.74, 2.30	32	228	1.22	0.69, 2.16	0.96	0.09
	GA/AA	18	139	1.14	0.59, 2.18	10	148	0.56	0.26, 1.20	23	146	1.27	0.69, 2.35	27	116	2.04	1.16, 3.74	0.017	
γ-Linolenic acid	GG	35	221	1.00	Referent	26	191	0.78	0.45, 1.35	37	196	1.04	0.62, 1.74	30	211	0.78	0.46, 1.33	0.77	0.42
	GA/AA	25	121	1.34	0.76, 2.36	17	151	0.70	0.37, 1.30	16	147	0.61	0.32, 1.16	20	130	0.86	0.47, 1.56	0.07	
Eicosadienoic acid	GG	37	209	1.00	Referent	27	200	0.74	0.43, 1.27	38	194	1.17	0.71, 1.93	26	217	0.68	0.39, 1.17	0.29	0.09
	GA/AA	14	134	0.62	0.32, 1.20	19	138	0.83	0.46, 1.52	21	151	0.80	0.45, 1.44	24	126	1.12	0.64, 1.99	0.06	
Dihomo-γ- linolenic acid	GG	40	213	1.00	Referent	29	200	0.76	0.45, 1.28	31	207	0.80	0.48, 1.34	28	100	0.73	0.43, 1.25	0.33	0.30
	GA/AA	17	131	0.70	0.38, 1.30	20	140	0.80	0.44, 1.43	18	132	0.74	0.40, 1.37	23	146	0.85	0.48, 1.51	0.66	
Arachidonic acid	GG	30	207	1.00	Referent	34	220	1.11	0.65, 1.89	36	204	1.29	0.76, 2.19	28	189	1.03	0.59, 1.82	0.62	0.036
	GA/AA	26	134	1.36	0.76, 2.41	21	124	1.24	0.68, 2.29	21	139	1.11	0.61, 2.03	10	152	0.49	0.23, 1.07	0.024	
Docosadienoic acid	GG	24	202	1.00	Referent	33	207	1.38	0.78, 2.44	33	207	1.44	0.81, 2.54	38	204	1.69	0.97, 2.94	0.05	0.53
	GA/AA	11	137	0.72	0.34, 1.53	19	135	1.29	0.68, 2.47	24	142	1.50	0.81, 2.77	24	135	1.67	0.90, 3.10	0.027	
Docosatetraenoic acid	GG	37	208	1.00	Referent	44	197	1.31	0.81, 2.14	21	203	0.60	0.34, 1.07	26	212	0.70	0.41, 1.22	0.06	0.84
	GA/AA	20	133	0.89	0.49, 1.61	29	142	1.19	0.69, 2.06	19	142	0.80	0.44, 1.46	10	132	0.46	0.22, 0.97	0.08	

Table continues

Table 5. Continued

Fatty Acids	*MPO*[c] Genotype	Quartile 1				Quartile 2				Quartile 3				Quartile 4				P_{trend}	$P_{interaction}$
		No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI		
Total *n*-6	GG	32	201	1.00	Referent	29	206	0.91	0.53, 1.57	38	198	1.28	0.76, 2.16	29	215	0.95	0.55, 1.64	0.97	0.74
	GA/AA	21	138	1.02	0.56, 1.86	15	138	0.72	0.37, 1.39	21	145	1.02	0.56, 1.86	21	128	1.15	0.63, 2.11	0.49	

Abbreviations: CARET, Carotene and Retinol Efficacy Trial; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PUFA, polyunsaturated fatty acid.

[a] Multivariate adjustment for age at enrollment (continuous), race (white, black, others), CARET randomization assignment (retinol plus β-carotene, placebo), family history of prostate cancer in first-degree relatives (yes, no), alcohol consumption (nondrinker, below median, at or above median, unknown), smoking status (current, former/never), smoking pack-years (<40, 40, <60, ≥60), and body mass index (continuous).

[b] Defined as stage III/IV tumors or Gleason score ≥7.

[c] The myeloperoxidase gene, *MPO*.

was observed between serum EPA + DHA percentages and aggressive prostate cancer risk. However, among men with the *MPO* GA/AA genotype, the direction of association was opposite. This risk difference by genetic variation in *MPO* was statistically significant ($P_{interaction} = 0.011$). The effect modification of the *MPO* genotype was also observed for docosapentaenoic acid (DPA (22:5*n*-3); $P_{interaction} = 0.013$), DHA only ($P_{interaction} = 0.028$), and total *n*-3 PUFAs ($P_{interaction} = 0.002$) illustrated in Figure 1. For *n*-6 PUFAs, the effect modification was observed for arachidonic acid ($P_{interaction} = 0.036$). In the secondary analysis (Web Table 4), the PUFA/*MPO* interactions remained significant for high-grade cancer. We observed a suggestive pattern that *MPO* modified the associations of EPA + DHA and total *n*-3 PUFAs with advanced stage prostate cancer risk, while there was no clear pattern of effect modification for lethal prostate cancer risk. In a sensitivity analysis, we additionally included serum α-linolenic acid, linoleic acid, and total *trans*-fatty acid percentages and α-tocopherol concentrations (all in quartiles) in the models because they are either metabolic precursors or correlated with individual PUFAs or lipid peroxidation (12, 23). The observed associations and effect modification remained unchanged (data not shown).

DISCUSSION

In this nested case-control study in CARET, the associations of serum EPA, DPA, DHA, total *n*-3 PUFAs, and arachidonic acid with aggressive prostate cancer were modified by the *MPO* G-463A polymorphism. We did not find any joint association of this polymorphism with serum *trans*-fatty acids. Our findings have important implications in the prevention of prostate cancer since EPA, DHA, and arachidonic acid are the most biologically relevant to signaling metabolic enzymes and inflammation (4). In addition, our data suggest that the effect modification is more relevant to aggressive prostate cancer compared with nonaggressive prostate cancer, as the former confers worse clinical outcome and should be the primary target of prostate cancer prevention. To our knowledge, this is the first study reporting the interaction between serum PUFAs and genetic variation in *MPO*. The interaction is consistent with previous analyses in CARET showing that high iron intake combined with the *MPO* GG genotype, both promoting oxidative stress, was associated with an increased risk of aggressive prostate cancer (11), and that high serum concentrations of α-tocopherol combined with the GA/AA genotypes, both lowering oxidative stress, decreased risk (12).

Two seemingly opposite mechanisms of oxidative stress and metabolic signaling of PUFAs potentially explain our findings. First, lipid peroxidation triggers myeloperoxidase located in neutrophils, monocytes, and some macrophages to generate endogenous free radicals (24) that damage prostate tissue (6, 7, 25). In addition, free radicals can directly react with PUFAs to form chlorohydrin, a substance that has higher polarity than the parent fatty acid and thus interrupts cell membrane structure, resulting in cell toxicity (9). Our analyses in CARET suggest that high *MPO* activity (the GG genotype), leading to high lipid peroxidation and free radical cellular concentrations, in conjunction with high

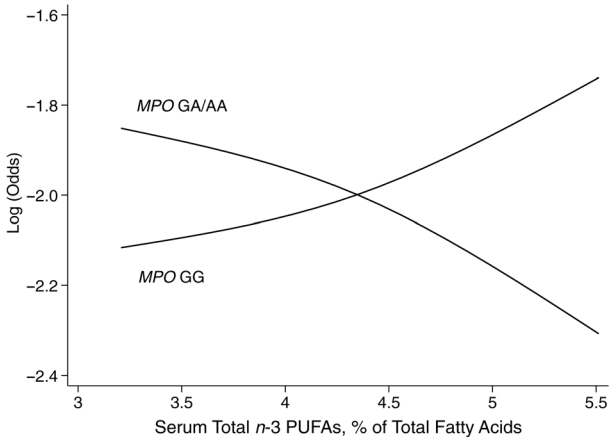

Figure 1. Predicted log(odds) of serum total *n*-3 polyunsaturated fatty acids (PUFAs) with aggressive prostate cancer by myeloperoxidase gene polymorphism (*MPO* G-463A) in the Carotene and Retinol Efficacy Trial, 1985–2003. Log(odds) were predicted from a logistic model containing a product term between the *MPO* genotype and serum total *n*-3 PUFAs as a continuous variable ($P_{interaction}$ = 0.028). The predicted log(odds) were fit by using a cubic spline with 4 knots (the midpoint of each quartile).

percentages of serum *n*-3 PUFAs may increase prostate cancer risk. On the contrary, our joint effect model suggests that, under the condition of low percentages of total *n*-3 PUFAs, the genotype conferring low *MPO* activity (GA/AA) was associated with a 2-fold increase in aggressive prostate risk compared with the GG genotype. Studies have suggested that absence of oxidative stress and the protection of *n*-3 PUFA may lower cell apoptosis (26, 27). Nevertheless, since our study participants might already have an elevated level of oxidative stress due to their history of smoking (28), this observation warrants replication among nonsmokers.

Compared with *n*-3 PUFAs, *n*-6 PUFAs are more proinflammatory by influencing cell cycle regulatory genes and promoting cyclooxygenase and lipoxygenase syntheses (5, 10). Arachidonic acid signals prostaglandin E_2, a major metabolite of cyclooxygenase-2 (29). In addition, after lipid peroxidation, *n*-6 PUFAs generate 4-hydroxy-2-nonenal, a cytotoxic aldehyde leading to DNA damage (5, 30). Epidemiologic findings of *n*-6 PUFAs in relation to prostate cancer are inconsistent (2, 23, 31, 32). For example, the Physicians' Health Study observed a positive association for dihomo-γ-linolenic acid in whole blood (23), while we found that dihomo-γ-linolenic acid and docosatetraenoic acid were inversely associated with nonaggressive and aggressive prostate cancer, respectively, Our observation has a biological rationale: Dihomo-γ-linolenic acid is a substrate for prostaglandin E_1, which is more antiinflammatory than prostaglandin E_2 (33). Also, docosatetraenoic acid is elongated from arachidonic acid; the replacement can lead to a lower level of inflammation. It is noted that the percentages of dihomo-γ-linolenic and docosatetraenoic acids in serum are relatively small and the number of comparisons has increased in order to estimate their associations. Thus, the significant findings should be interpreted with caution.

Our findings on interaction between *n*-6 PUFAs and *MPO* genotypes are parallel to those in the Alpha-Tocopherol Beta-Carotene Study. That study also recruited smokers and found that serum linoleic acid was inversely associated with prostate cancer risk only among men who received high-dose α-tocopherol supplements that lower oxidative stress and lipid peroxidation (for quartiles 4 vs. 1: OR = 0.17, 95% CI: 0.04, 0.68) (31). Our study found an inverse association of serum arachidonic acid with aggressive prostate cancer among men with the *MPO* GA/AA genotypes (related to low endogenous free radicals). These 2 observations suggest that the inflammatory response of *n*-6 PUFAs may depend on the oxidative stress level.

A major strength of our study is its nested case-control design, which measured fatty acids in serum collected on average 7 years prior to prostate cancer diagnosis. Also, a large number of cases enabled us to estimate risks for both nonaggressive and aggressive prostate cancer. The grade and stage of prostate cancer were confirmed by medical records or cancer registry files. Nevertheless, limitations to this study should be noted. First, it may not be appropriate to generalize our study findings to other populations because CARET participants were heavy smokers and/or had occupational asbestos exposure. Characteristics related to lipid peroxidation levels and expression of reactive oxygen species detoxifying enzymes in the study population may be different from those in other populations. Second, the long-term systematic or random variations of serum fatty acids may have biased our risk estimates toward the null since we measured them at one point in time. Third, we conducted statistical tests in individual components of *n*-3 and *n*-6 PUFAs, a strategy that may lead to an increase in type I error. However, since the hypothesis of PUFAs/*MPO* interaction was a priori and we found significant interactions in several *n*-3 and *n*-6 PUFAs with biological relevance, the probability of our findings due to chance alone was low (34). Finally, there is lack of consensus on defining aggressive prostate cancer. The Gleason pattern, for example, 4 + 3, was missing for most cases because of incomplete information on the pathology reports and medical records. We therefore used a Gleason score ≥8 to define "high-grade" prostate cancer and observed a consistent pattern of effect modification. However, we cannot make a clear conclusion for "advanced stage" or "lethal" prostate cancer because of the small numbers of patients with these tumors.

In conclusion, in this population of heavy smokers, the genetic variation in *MPO* G-463A is an important effect modifier of the association of *n*-3 and *n*-6 PUFAs, including EPA, DPA, DHA, and arachidonic acid, with aggressive prostate cancer. The longtime hypothesized beneficial and adverse effects of PUFAs on prostate cancer risk should be reevaluated, because they may depend on the activity of oxidative stress-regulatory enzymes.

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Author affiliations: Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington

(Ting-Yuan David Cheng, Irena B. King, Matt J. Barnett, Mark D. Thornquist, Gary E. Goodman, Marian L. Neuhouser); Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington (Ting-Yuan David Cheng, Marian L. Neuhouser); Department of Internal Medicine, School of Medicine, University of New Mexico, Albuquerque, New Mexico (Irena B. King); and Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, New York (Christine B. Ambrosone).

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