Dietary lycopene intake and risk of prostate cancer defined by ERG protein expression[1]

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ABSTRACT

Background: There is limited evidence that supports etiologically distinct molecular subtypes of prostate cancer, the identification of which may improve prevention. Given their antioxidant properties, we hypothesized that lycopene and tomato sauce may be especially protective against diseases harboring the common gene fusion transmembrane protease, serine 2 (*TMPRSS2*):v-ets avian erythroblastosis virus E26 oncogene homolog (*ERG*).

Objective: We aimed to examine associations between estimated lycopene and tomato sauce intake and the risk of prostate cancer defined by ERG protein expression subtype.

Design: Our study population consisted of a prospective cohort of 46,719 men from the Health Professionals Follow-Up Study. *TMPRSS2:ERG* was assessed by ERG immunohistochemistry on tumor tissue microarrays constructed from radical prostatectomy specimens. We used multivariable competing risk models to calculate HRs and 95% CIs for the risk of ERG-positive and, separately, ERG-negative disease. We implemented inverse probability weighting to account for evaluating ERG status only in surgically treated cases.

Results: During 23 y of follow-up, 5543 men were diagnosed with prostate cancer, among whom 884 were assayed for ERG (426 ERG-positive). With inclusion of only the latter cases, increasing cumulative average tomato sauce intake was associated with a decreased risk of prostate cancer overall (≥2 servings/wk compared with <1 serving/mo; multivariable HR: 0.70; 95% CI: 0.52, 0.95; *P*-trend = 0.002). With respect to molecular subtypes, cumulative average tomato sauce intake was associated with a decreased risk of ERG-positive disease (HR: 0.54; 95% CI: 0.37, 0.81; *P*-trend = 0.004) but not with ERG-negative disease (HR: 0.96; 95% CI: 0.62, 1.50; *P*-trend = 0.10) (*P*-heterogeneity = 0.04). Increasing quintiles of lycopene intake were associated with a decreased risk of both subtypes (*P*-heterogeneity = 0.79). Inverse probability weighting did not materially change the results.

Conclusions: Our results lend some support to the hypothesis that prostate cancers that harbor *TMPRSS2:ERG* may be etiologically distinct from fusion-negative cancers. In particular, tomato sauce consumption may play a role in reducing *TMPRSS2:ERG*-positive disease. *Am J Clin Nutr* 2016;103:851–60.

Keywords: ERG protein expression, *TMPRSS2:ERG*, lycopene, prostate cancer, tomato sauce

INTRODUCTION

Prostate cancer is marked by genetic complexity (1–3), including point mutations, chromosomal rearrangements, and gene fusion events, the most common of which is the transmembrane protease, serine 2 (*TMPRSS2*):v-ets avian erythroblastosis virus E26 oncogene homolog (*ERG*)[13] fusion (4). Few studies have considered possible etiologic differences between distinct molecular subtypes of disease. The identification of risk factors for molecular subtypes of prostate cancer has the potential to improve opportunities for prevention of a disease for which modifiable risk factors have remained elusive.

TMPRSS2:ERG occurs in the tumors of half of patients with prostate cancer (5), translating to >100,000 new cases of fusion-positive cancer in the United States each year (6). Experimental and clinical evidence suggests that cancers with the fusion define a distinct subgroup of prostate cancers with respect to phenotypic changes and disease progression (5). From an etiologic perspective,

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[13] Abbreviations used: ERG, v-ets avian erythroblastosis virus E26 oncogene homolog; FFQ, food-frequency questionnaire; HPFS, Health Professionals Follow-Up Study; IGF-I, insulin-like growth factor I; PSA, prostate-specific antigen; RP, radical prostatectomy; TMA, tissue microarray; TMPRSS2, transmembrane protease, serine 2.

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however, there are scant epidemiologic data regarding differential risk factors for *TMPRSS2:ERG*-positive and -negative tumors. Only recently, one population-based case-control study provided some evidence for a differential association between obesity and the risk of cancers that are positive compared with negative for *TMPRSS2:ERG* (7).

This article focuses on the carotenoid lycopene and tomato sauce for its substantial contribution to lycopene intake. Many analyses, including in the HPFS (Health Professionals Follow-Up Study), have shown increasing lycopene and tomato sauce intake to be inversely associated with prostate cancer risk (8–10). Lycopene is the most efficient among carotenoids in quenching singlet oxygen molecules (11–13), and it may also interfere with reactions initiated by free radicals (14, 15). These exceptional antioxidant properties and the accumulation of lycopene at high concentrations in the prostate (16–19) are a likely basis for the role of lycopene in protecting against prostate cancer. That antioxidants result in a reduction in DNA damage (17, 20–23) becomes intriguing in the context of *TMPRSS2:ERG*, which results from the ineffective repair of DNA double-strand breaks (24–26).

In this prospective cohort study within the HPFS, we examined the hypothesis that lycopene and tomato sauce intakes are associated with a lower risk of *TMPRSS2:ERG*-positive prostate cancer more so than of *TMPRSS2:ERG*-negative prostate cancer. We incorporated novel statistical methods using inverse probability weighting to account for sampling prostate cancer cases treated with radical prostatectomy (RP).

METHODS

Study population

The HPFS is an ongoing prospective cohort of 51,529 male health professionals in the United States, aged 40–75 y at enrollment in 1986. Participants responded to a baseline questionnaire with regard to their medical histories and known or suspected contributors to cancer and other chronic diseases. Follow-up questionnaires have been mailed every 2 y to update information on lifestyle factors and to identify newly diagnosed health outcomes.

For these analyses, we excluded men who reported cancers other than nonmelanoma skin cancer at baseline in 1986 (n = 2087). We also excluded men who reported implausible caloric intakes (<800 or >4200 kcal/d), who left >70 food items blank (n = 1524), or who did not report their consumption of tomato sauce (n = 1167) on the baseline food-frequency questionnaire (FFQ). Last, we excluded men who were missing a date of birth (n = 32). The remaining 46,719 men comprised the study population for these analyses.

The institutional review boards at the Harvard T.H. Chan School of Public Health and Partners Health Care approved this study. Written informed consent was obtained from each subject.

Assessment of dietary intake

Dietary intake, including that of tomato sauce and other foods contributing to lycopene intake, was estimated via a semiquantitative FFQ, described in detail elsewhere (27), administered every 4 y since 1986. For each item listed on the FFQ, a commonly used unit or portion size was specified, and participants were asked how often, on average, over the past year, they had consumed that amount of each food. Participants could choose from 9 possible frequencies, ranging from never to ≥6 times/d.

Food items listed on the FFQ that contributed to lycopene intake included tomatoes, tomato sauce, tomato juice, pizza, salsa, picante or taco sauce, ketchup or red chili sauce, watermelon, and pink grapefruit. Total lycopene intake was computed by multiplying the consumption frequency of each unit of the food items by the lycopene content of the specified portions by using composition values from the USDA sources supplemented with other data (28–30). Mean correlation coefficients between intakes determined by two 1-wk diet records and the FFQ (adjusted for week-to-week variation in the diet records) were, on average, 0.64 for total carotene (27) and 0.37 for tomato sauce (31). The correlation between the computed dietary intake of lycopene and plasma concentration of lycopene adjusted for age, BMI, and plasma lipids was 0.46 (32). Among specific food items, tomato sauce had the strongest correlation with blood lycopene (r = 0.37) (32).

Ascertainment of prostate cancer cases and clinical data

Prostate cancer cases were initially identified by self-report or participants' next of kin and confirmed by medical record and pathology report. Given the high accuracy of reporting among men with available medical records, these analyses included the 9% of cases indicated only by self-report or death certificates. Deaths were ascertained via reports from family members and inspection of the National Death Index. Follow-up for mortality was >98% complete.

The study team reviewed records to abstract information about tumor stage, Gleason score, and prostate-specific antigen (PSA) level at diagnosis, as well as treatments. To reduce detection bias, we censored men who were diagnosed with stage T1a cancers (n = 257), which are discovered incidentally during treatment of benign prostatic hypertrophy (33). Prostate tissue was available for prostate cancer cases through July 2009; we thus ended follow-up at that time. In total, 5543 prostate cancer cases were diagnosed during the study period.

Tumor tissue cohort and immunohistochemistry

We retrieved archival prostate tumor tissue from men who underwent RP (95%) or transurethral resection of the prostate (5%). The retrieval process was previously described (34). Among the 5543 cases, we undertook biomarker analysis for 884 cases with formalin-fixed paraffin-embedded RP specimens. Study pathologists reviewed hematoxylin-and-eosin slides to provide uniform Gleason grade and other histopathologic features and to select areas of tumor for construction of tumor tissue microarrays (TMAs) (35). We constructed TMAs by taking at least three 0.6-mm tumor cores from the primary nodule or nodule with the highest Gleason grade and transferring them to a recipient block (36).

We used immunohistochemistry of ERG protein expression on TMAs as a proxy measure of *TMPRSS2:ERG* status. The method has been shown to have high concordance with fusion status assessed by alternate methods (37–39). Details of the assessment

were described previously (40). Briefly, ERG antisera (1:100, Clone ID EPR3864; Epitomics) were applied to 0.5-μm TMA sections and visualization of ERG was accomplished by using the 3, 3-diaminobenzidine substrate kit (Vector Laboratories). A single pathologist scored each case as ERG-positive if at least 1 TMA core had positive ERG staining within prostate cancer epithelial cells. Of cases that were positive for ERG on at least 1 core, 85% stained positive for ERG in all cores.

Dietary exposures

We assessed estimated total lycopene and tomato sauce intakes as separate exposures. Lycopene intake was adjusted for total energy intake by using residuals from a regression analysis (41) and categorized into quintiles. Tomato sauce intake was categorized into prespecified FFQ categories and adjusted for energy intake by inclusion in multivariable models. We first used cumulative average intakes to minimize within-person random variation and computed an assessment of long-term intake using all available questionnaires (42). By using this categorization, we related intake from the 1986 questionnaire to cancer incidence from 1986 to 1990, average intake from the 1986 and 1990 questionnaires to cancer incidence from 1990 to 1994, and so forth. Data from the previous FFQ were carried forward to the next time period for participants with incomplete FFQ information after baseline. Second, we used quantiles of baseline exposure to allow for a maximum induction period between exposure and period of risk.

Statistical analysis

Inverse probability weighting

ERG status was evaluated in 884 men with available tissue out of 2300 total men who were treated with RP. This subset of patients was diagnosed at a more localized stage, had tumors with lower Gleason scores, had lower PSA levels, and were more often diagnosed in earlier years relative to cases in the HPFS who received other treatments (e.g., external beam radiation) (n = 3243). To account for the potential bias due to overselecting surgery patients, we implemented inverse probability weighting to validly estimate the association between exposures and prostate cancer incidence by ERG expression subtype. We applied weights, in each time period, equal to 1 for all subjects who did not develop cancer and equal to zero for patients who developed cancer but who did not have RP tissue available. For patients who had tissue available for assay, we applied weights that accounted for clinical characteristics at and timing of diagnosis. Whenever clinical stage (n = 664), Gleason score (n = 1099), or PSA (n = 1037) were not available, we implemented 2 methods to deal with the missingness when creating the weights. For our primary analyses, we used a missing indicator for men without values of stage, Gleason score, and PSA at diagnosis. In secondary analyses, we created and implemented weights in which we replaced missing data by the most common value among men with data. Results were comparable for the 2 methods, so we only present results from the former.

Cox models and competing risks

Participants contributed person-time from the date on which they returned the baseline questionnaire until prostate cancer diagnosis, death, or end of follow-up. We ran Cox proportional hazards models adjusted for age and calendar time to assess associations between dietary exposures and prostate cancer risk overall. In addition, multivariable models were adjusted for race (white, African American, Asian American, or other), height ($\leq$68, $>$68–70, $>$70–72, or $>$72 inches), BMI at age 21 (in kg/m^2; $<$20, 20 to $<$22.5, 22.5 to $<$25, or $\geq$25), current BMI ($<$21, 21 to $<$23, 23 to $<$25, 25 to $<$27.5, 27.5 to $<$30, or $\geq$30), vigorous physical activity (quintiles of metabolic equivalent task hours/wk), smoking status (never, former/quit $>$10 y ago, former/quit $\leq$10 y ago, or current), diabetes (yes or no), family history of prostate cancer in father or brother (yes or no), PSA testing in the previous period (yes; no, lagged by one period to avoid counting diagnostic PSA tests as screening; collected from 1994 on; cumulative average models only), use of multivitamins (yes or no), total calories (continuous), and intakes of calcium, α-linolenic acid, supplemental vitamin E, alcohol (quintiles), and coffee (none, $<$1, 1 to $<$2, 2 to $<$3, or $\geq$3 cups/d). For cumulative average models, covariates other than height, race, BMI at age 21, and family history of prostate cancer were updated in each questionnaire cycle. The results from simple and multivariable models were comparable; we thus present results from the latter models only. For all of the analyses, we conducted linear trend tests across quantiles by modeling their median values as continuous variables.

Next, we implemented an extension of Cox modeling as described by Lunn and McNeil (43) that allows for risk factor associations to vary by subtype. The method has been previously described in detail (44). Briefly, we augmented the data to create 2 records for each subject in each questionnaire cycle, one each for ERG-positive and ERG-negative disease. For evaluation of ERG-positive cancer, cases diagnosed with ERG-negative disease were censored at diagnosis, and vice-versa. Men with prostate cancer for whom ERG status was not assessed were also censored at diagnosis. We fit a model that allowed for estimating HRs for ERG-positive cancer and, separately, for ERG-negative cancer compared with no cancer. We tested for heterogeneity across these HRs using a likelihood ratio test (45).

For the models of prostate cancer overall and for those assessing subtype-specific associations, we performed both unweighted and weighted analyses. For the latter, we weighted each individual by the inverse probability weights described above. To explore possible confounding by PSA screening, we ran analyses stratified by time period, examining associations separately for pre–PSA-era diagnoses (1986–1993) and PSA-era diagnoses (1994–2009). Finally, in exploratory unweighted analyses, we evaluated the risk of advanced prostate cancer (stage T3b or higher at diagnosis, development of metastases during follow-up, or death from prostate cancer) overall and by ERG status.

Analyses were conducted by using SAS version 9.4 (SAS Institute). Tests were 2-sided, with $P < 0.05$ considered to be significant.

RESULTS

At baseline in 1986, lycopene intakes ranged from a median of 2764 to 13,573 μg/d across extreme quintiles (**Table 1**). Men with the lowest intake were slightly older than men with higher intakes. Relative to men in the lowest quintile, men in the highest quintile were more physically active and consumed more

TABLE 1
Age-adjusted characteristics of the HPFS at baseline in 1986 (unless otherwise noted) according to quintiles of energy-adjusted dietary lycopene intake[1]

	Baseline dietary lycopene intake quintile, range (median)				
	≤3861 (2764) μg/d	3862–5439 (4670) μg/d	5440–7196 (6258) μg/d	7197–10,261 (8440) μg/d	≥10,262 (13,573) μg/d
n	9707	9345	9242	9183	9242
Age,[2] y	56.9 ± 9.8[3]	54.4 ± 9.6	53.6 ± 9.5	53.2 ± 9.5	53.4 ± 9.5
Height, inches	70.2 ± 2.8	70.2 ± 2.8	70.1 ± 2.8	70.0 ± 2.9	70.0 ± 3.0
BMI at age 21, kg/m^2	22.9 ± 3.1	22.9 ± 2.9	23.0 ± 2.9	23.2 ± 3.0	23.2 ± 3.2
BMI, kg/m^2	25.3 ± 3.4	25.4 ± 3.2	25.5 ± 3.2	25.6 ± 3.3	25.8 ± 3.6
White, %	94.4	95.9	96.2	96.0	95.7
Family history of prostate cancer, %	12.9	11.9	12.3	11.9	10.9
Diabetes, %	2.9	2.6	3.1	3.3	3.8
Top quintile of physical activity (≥28.5 MET-h/wk), %	12.5	13.2	15.6	16.5	18.1
Smoking status, %					
Never	45.7	46.5	46.5	48.1	46.1
Past, quit >10 y before baseline	28.5	30.4	30.9	30.6	32.0
Past, quit ≤10 y before baseline	13.4	12.9	13.0	12.5	12.9
Current	12.4	10.2	9.7	8.7	9.0
Multivitamin use, %	40.9	41.1	41.9	41.8	42.2
Had PSA test,[4] %					
1994	36.7	37.6	38.3	39.3	37.2
2004	58.9	62.5	62.3	62.2	61.1
Nutrient and food intakes					
Total energy, kcal/d	2078 ± 673	2061 ± 587	1944 ± 566	1907 ± 635	1951 ± 615
Calcium, mg/d	906 ± 455	899 ± 414	895 ± 409	893 ± 420	888 ± 421
α-Linolenic acid, g/d	1.0 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3
Supplemental vitamin E, mg/d	35.2 ± 81.0	34.5 ± 81.0	37.4 ± 84.2	39.9 ± 87.0	42.3 ± 90.5
Alcohol, g/d	12.5 ± 17.8	11.8 ± 15.8	11.0 ± 14.5	10.5 ± 14.0	10.7 ± 14.5
Tomatoes,[5] servings/wk	1.4 ± 1.4	2.2 ± 1.8	2.7 ± 2.1	3.2 ± 2.5	4.2 ± 4.0
Tomato juice,[5] servings/wk	0.1 ± 0.2	0.2 ± 0.3	0.3 ± 0.4	0.5 ± 0.8	1.6 ± 2.6
Tomato sauce,[5] servings/wk	0.3 ± 0.3	0.6 ± 0.3	0.7 ± 0.4	1.0 ± 0.8	2.2 ± 1.9
Pizza,[5] servings/wk	0.3 ± 0.3	0.4 ± 0.4	0.6 ± 0.5	0.7 ± 0.7	0.9 ± 1.1
Coffee, cups/d	1.5 ± 1.7	1.4 ± 1.6	1.3 ± 1.6	1.2 ± 1.5	1.2 ± 1.5

[1]1 inch = 0.0254 m; 1 cup = 236.588 mL. HPFS, Health Professionals Follow-Up Study; MET-h, metabolic equivalent task hours; PSA, prostate-specific antigen.
[2]Not adjusted for age.
[3]Mean ± SD (all such values).
[4]Reported having a PSA test in the 2 y before the questionnaire date.
[5]1 serving = 1 tomato; 1 small glass of tomato juice; 0.5 cup (118.294 mL) tomato sauce; 2 slices of pizza.

supplemental vitamin E, less alcohol, and more tomato-based products. Baseline tomato sauce intake ranged from never to a median of 3 servings/wk in the highest category of intake.

During 23 y of follow-up, we identified 5543 total prostate cancer cases (**Table 2**). Among them, 2300 were treated with RP, of whom 884 (15.9% of all cases) were assayed for ERG. Among men treated with RP, those without tissue available were more likely to be diagnosed in later years. As a result, they were also more likely to consume slightly higher amounts of lycopene, the intake of which increased over time in our cohort. Lifestyle and demographic factors were otherwise similar for all men treated with RP. Relative to such men, cases who were not treated with RP were more likely to be older at the time of diagnosis and to be diagnosed with higher grade and stage disease and PSA levels. They also had a higher prevalence of diabetes and were less likely to never have been smokers. In addition, data on their clinical characteristics were less likely to be available at diagnosis.

Quintiles of cumulative average lycopene intake were associated with a slightly reduced risk of prostate cancer overall after adjustment for potential confounding variables (**Table 3**). Similarly, cumulative average tomato sauce intake was inversely associated with risk. Restricting analyses to include only cases in whom ERG had been assayed, comparable HRs were more strongly inverse for both lycopene intake and tomato sauce intake. Applying inverse probability weights to account for the distinct distribution of clinical factors in the group assayed for ERG did not materially change the results. Associations for baseline intakes of exposures were similar, if slightly stronger.

Cumulative average lycopene intake was associated with a reduced risk of both ERG-positive prostate cancer and ERG-negative prostate cancer (**Table 4**). Differences between the estimates for ERG-positive and ERG-negative disease were not significant for either cumulative average (*P*-heterogeneity = 0.79) or baseline (*P*-heterogeneity = 0.51) lycopene intake. Cumulative average tomato sauce intake was associated with a reduced risk of ERG-positive cancer but had no association with ERG-negative cancer (*P*-heterogeneity = 0.04). Similarly, estimates for baseline tomato sauce intake and the risk of ERG-positive and -negative cancers differed significantly (*P*-heterogeneity = 0.02), although both estimates for the highest

TABLE 2
Characteristics of participants with prostate cancer in the HPFS at the time of diagnosis (1986–2005), by treatment and ERG status[1]

	Participants treated with RP			Participants treated otherwise
Characteristic	ERG-positive	ERG-negative	ERG status unavailable	ERG status unavailable
n	426	458	1416	3243
Year of diagnosis, %				
1986–1990	8.0	5.9	8.1	8.2
1991–1995	35.7	31.2	25.7	22.1
1996–2000	30.3	32.1	24.1	26.1
2000–2005	16.9	21.0	23.3	27.6
2006–2009	9.2	9.8	18.8	16.1
Age, y	65.1 ± 6.1[2]	65.8 ± 5.8	65.8 ± 5.9	72.9 ± 6.9
PSA,[3] ng/mL	9.7 ± 11.6	10.4 ± 12.6	11.7 ± 112	20.8 ± 155
Missing, %	7.3	8.5	8.9	25.9
Had screening PSA test, %	47.0	48.7	50.7	51.0
Biopsy Gleason score, %				
2–6[3]	66.9	63.6	66.4	56.9
7[3]	26.8	26.4	27.0	28.2
8–10[3]	6.3	10.1	6.6	14.9
Missing	9.9	11.4	15.9	24.1
Clinical stage, %				
T1/T2[3]	94.6	95.4	95.6	87.7
T3[3]	4.5	3.1	2.7	3.9
T4/N1/M1[3]	0.9	1.5	1.7	8.4
Missing	0.0	0.0	2.1	17.8
BMI, kg/m^2	25.7 ± 3.5	26.1 ± 3.2	25.7 ± 3.1	25.9 ± 3.7
Diabetes, %	4.9	4.6	4.7	9.1
Top quintile of physical activity (≥28.5 MET-h/wk), %	13.6	14.7	17.2	12.8
Smoking status, %				
Never	50.7	50.7	48.0	44.1
Past, quit >10 y before diagnosis	32.0	34.3	36.8	38.8
Past, quit ≤10 y before diagnosis	11.5	8.9	10.9	11.9
Current	5.9	6.2	4.3	5.2
Multivitamin use, %	47.7	48.0	50.8	52.7
Nutrient and food intakes				
Total energy, kcal/d	1993 ± 586	1981 ± 584	1945 ± 598	1975 ± 623
Lycopene, μg/d	7105 ± 4925	7462 ± 6203	7601 ± 6410	7432 ± 6859
Calcium, mg/d	1011 ± 479	988 ± 444	1015 ± 487	1053 ± 506
α-Linolenic acid, g/d	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.5	1.2 ± 0.5
Supplemental vitamin E, mg/d	63.7 ± 104	68.4 ± 105	68.2 ± 113	74.1 ± 118
Alcohol, g/d	12.1 ± 14.8	12.9 ± 16.2	11.5 ± 13.6	12.0 ± 16.0
Tomatoes,[4] servings/wk	2.5 ± 2.2	2.5 ± 2.1	2.8 ± 2.6	3.0 ± 3.3
Tomato juice,[4] servings/wk	0.6 ± 1.2	0.5 ± 1.4	0.6 ± 1.4	0.7 ± 1.9
Tomato sauce,[4] servings/wk	0.9 ± 1.1	1.1 ± 1.1	1.1 ± 1.6	1.0 ± 1.4
Pizza,[4] servings/wk	0.5 ± 0.6	0.5 ± 0.6	0.5 ± 0.7	0.4 ± 0.7
Coffee, cups/d	1.2 ± 1.5	1.2 ± 1.5	1.2 ± 1.4	1.1 ± 1.4

[1]ERG, v-ets avian erythroblastosis virus E26 oncogene homolog; HPFS, Health Professionals Follow-Up Study; MET-h, metabolic equivalent task hours; PSA, prostate-specific antigen; RP, radical prostatectomy.
[2]Mean ± SD (all such values).
[3]Among those with data available.
[4]1 serving = 1 tomato; 1 small glass of tomato juice; 0.5 cup (118.294 mL) tomato sauce; 2 slices of pizza.

intake category were significantly protective. The application of inverse probability weights did not materially change the results.

Analyses stratified by PSA era were not as well powered to detect associations or heterogeneity. However, the exposures were generally more strongly inversely associated with prostate cancer overall in the pre-PSA era than in the PSA era. Cumulative average lycopene and tomato sauce intakes were more strongly inversely associated with ERG-positive prostate cancer in the pre-PSA era than in the PSA era. Baseline dietary intakes were more strongly associated with ERG-negative prostate cancer in the PSA era than in the pre-PSA era (data not shown). Analyses of advanced prostate cancer were poorly powered but generally resulted in more strongly inverse point estimates relative to analyses of all prostate cancer and showed similar patterns of heterogeneity by ERG status (data not shown).

DISCUSSION

In this integrative molecular epidemiology study, we confirmed that lycopene and tomato sauce intakes were inversely

TABLE 3
HRs (95% CIs) for lycopene and tomato sauce intakes and risk of prostate cancer overall: HPFS[1]

	All cases		RP cases assayed for ERG status only		
				HR (95% CI)	
	Cases, *n*	HR (95% CI)	*n*	Unweighted	Weighted
Lycopene intake					
Cumulative average (median)					
Q1 (3247 μg/d)	1219	1.00 (ref)	187	1.00 (ref)	1.00 (ref)
Q2 (5085 μg/d)	1199	1.05 (0.97, 1.14)	204	1.03 (0.84, 1.25)	1.05 (0.85, 1.29)
Q3 (6652 μg/d)	1102	0.98 (0.90, 1.06)	192	0.95 (0.78, 1.17)	1.03 (0.82, 1.28)
Q4 (8711 μg/d)	1034	0.95 (0.87, 1.03)	161	0.79 (0.64, 0.99)	0.78 (0.62, 0.98)
Q5 (12,941 μg/d)	989	0.95 (0.87, 1.03)	140	0.72 (0.58, 0.91)	0.72 (0.57, 0.91)
P-trend		0.04		<0.001	<0.001
Baseline (median)					
Q1 (2764 μg/d)	1292	1.00 (ref)	217	1.00 (ref)	1.00 (ref)
Q2 (4670 μg/d)	1148	1.00 (0.92, 1.08)	205	0.96 (0.80, 1.17)	0.92 (0.75, 1.13)
Q3 (6258 μg/d)	1108	0.99 (0.91, 1.08)	174	0.82 (0.67, 1.00)	0.80 (0.64, 0.99)
Q4 (8440 μg/d)	1044	0.96 (0.89, 1.05)	167	0.80 (0.65, 0.99)	0.79 (0.63, 0.99)
Q5 (13,573 μg/d)	951	0.88 (0.81, 0.96)	121	0.59 (0.47, 0.74)	0.57 (0.44, 0.72)
P-trend		0.001		<0.001	<0.001
Tomato sauce intake[2]					
Cumulative average					
<1 serving/mo	681	1.00 (ref)	91	1.00 (ref)	1.00 (ref)
1 serving/mo to <1 serving/wk	2899	0.97 (0.89, 1.06)	484	1.01 (0.81, 1.28)	1.11 (0.87, 1.42)
1 serving/wk to <2 servings/wk	1304	0.96 (0.87, 1.06)	213	0.89 (0.69, 1.14)	0.92 (0.70, 1.21)
≥2 servings/wk	659	0.89 (0.79, 0.99)	96	0.70 (0.52, 0.95)	0.71 (0.52, 0.98)
P-trend		0.02		0.002	<0.001
Baseline					
<1 serving/mo	1097	1.00 (ref)	168	1.00 (ref)	1.00 (ref)
1 serving/mo to <1 serving/wk	2171	0.95 (0.89, 1.03)	350	0.88 (0.73, 1.07)	0.90 (0.73, 1.11)
1 serving/wk to <2 servings/wk	1649	0.97 (0.89, 1.05)	294	0.87 (0.71, 1.06)	0.86 (0.70, 1.07)
≥2 servings/wk	626	0.84 (0.76, 0.93)	72	0.49 (0.36, 0.65)	0.48 (0.35, 0.67)
P-trend		0.001		<0.001	<0.001

[1]Results are from Cox proportional hazards models. All of the models adjusted for age, calendar time, race (white, African American, Asian American, or other), height (≤68, >68–70, >70–72, or >72 inches), BMI at age 21 y (kg/m^2; <20, 20 to <22.5, 22.5 to <25, or ≥25), current BMI (<21, 21 to <23, 23 to <25, 25 to <27.5, 27.5 to <30, or ≥30), vigorous physical activity (quintiles of metabolic equivalent task hours/wk), smoking (never, former/quit >10 y ago, former/quit ≤10 y ago, or current), diabetes (yes or no), family history of prostate cancer in father or brother (yes or no), PSA testing (yes; no, lagged by one period to avoid counting diagnostic PSA tests as screening; cumulative average models only), use of multivitamins (yes or no), total calories (continuous), and intakes of calcium, α-linolenic acid, supplemental vitamin E, alcohol (quintiles), and coffee (none, <1, 1 to <2, 2 to <3, or ≥3 cups/d). ERG, v-ets avian erythroblastosis virus E26 oncogene homolog; HPFS, Health Professionals Follow-Up Study; PSA, prostate-specific antigen; Q, quintile; ref, reference; RP, radical prostatectomy.

[2]1 serving = 0.5 cup (118.294 mL) tomato sauce.

associated with prostate cancer overall. We found that tomato sauce intake was more strongly associated with ERG-positive than with ERG-negative disease but found only weak evidence that lycopene intake may be differentially associated with the 2 subtypes.

Genomic rearrangements result from the ineffective repair of DNA double-strand breaks (24–26). The double-strand breaks that result in *TMPRSS2:ERG* have been shown to involve synergism between increased androgen receptor signaling and genotoxic stress (46–48). Lycopene reduces both (17, 49–56), and culturing prostate cancer cells with lycopene inhibits strand breaks (57). Our findings of inverse associations between exposures and ERG-positive disease lend some support to our a priori hypothesis that lycopene and tomato sauce may protect against the strand breaks that result in ERG-positive cancer.

It was unanticipated that analyses of lycopene and tomato sauce would return different results with respect to associations by ERG status. FFQ intakes of lycopene and tomato sauce were relatively equal surrogates of plasma lycopene in our data (32), and they showed similar magnitudes of association with prostate cancer overall. Given that analyses by ERG status used a smaller number of cases than analyses of prostate cancer overall, chance could have played a role in the different results across exposures. It is also possible that tomato sauce has components other than lycopene that contribute to a reduced risk of ERG-positive disease. Tomatoes contain phytochemicals beyond lycopene that may reduce oxidative stress, among them other carotenoids, polyphenols, potassium, folate, ascorbic acid, and tocopherols (58–60). Two experimental studies in rodent models found that tomato powder more effectively inhibited prostate carcinogenesis than lycopene

TABLE 4
HRs (95% CIs) for lycopene and tomato sauce and risk of ERG-positive and ERG-negative prostate cancer: HPFS[1]

	RP cases assayed for ERG status only					
			Unweighted HR (95% CI)		Weighted HR (95% CI)	
	ERG-positive cases, *n*	ERG-negative cases, *n*	ERG-positive	ERG-negative	ERG-positive	ERG-negative
Lycopene intake						
Cumulative average (median)						
Q1 (3247 μg/d)	97	90	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Q2 (5085 μg/d)	98	106	0.96 (0.73, 1.27)	1.09 (0.82, 1.45)	0.99 (0.74, 1.33)	1.11 (0.83, 1.49)
Q3 (6652 μg/d)	87	105	0.84 (0.63, 1.12)	1.07 (0.81, 1.43)	0.84 (0.62, 1.15)	1.23 (0.90, 1.67)
Q4 (8711 μg/d)	80	81	0.76 (0.56, 1.03)	0.83 (0.61, 1.12)	0.75 (0.55, 1.03)	0.81 (0.59, 1.11)
Q5 (12,941 μg/d)	64	76	0.65 (0.47, 0.89)	0.80 (0.59, 1.09)	0.62 (0.44, 0.86)	0.83 (0.60, 1.14)
P-trend			0.003	0.03	0.001	0.04
P-heterogeneity			0.79		0.40	
Baseline (median)						
Q1 (2764 μg/d)	109	108	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Q2 (4670 μg/d)	106	99	1.00 (0.77, 1.31)	0.93 (0.70, 1.22)	0.94 (0.71, 1.25)	0.90 (0.67, 1.21)
Q3 (6258 μg/d)	79	95	0.74 (0.55, 0.99)	0.90 (0.68, 1.19)	0.70 (0.51, 0.97)	0.89 (0.65, 1.20)
Q4 (8440 μg/d)	73	94	0.71 (0.52, 0.95)	0.90 (0.68, 1.19)	0.72 (0.52, 1.01)	0.85 (0.63, 1.15)
Q5 (13,573 μg/d)	59	62	0.58 (0.42, 0.80)	0.60 (0.44, 0.83)	0.52 (0.37, 0.73)	0.61 (0.44, 0.86)
P-trend			<0.001	0.001	<0.001	0.004
P-heterogeneity			0.51		0.64	
Tomato sauce intake[2]						
Cumulative average						
<1 serving/mo	56	35	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1 serving/mo to <1 serving/wk	233	251	0.80 (0.59, 1.08)	1.35 (0.95, 1.93)	0.88 (0.64, 1.21)	1.45 (0.99, 2.11)
1 serving/wk to <2 servings/wk	92	121	0.62 (0.45, 0.88)	1.31 (0.89, 1.92)	0.66 (0.46, 0.94)	1.32 (0.88, 1.98)
≥2 servings/wk	45	51	0.54 (0.37, 0.81)	0.96 (0.62, 1.50)	0.58 (0.38, 0.88)	0.92 (0.58, 1.46)
P-trend			0.004	0.10	0.005	0.02
P-heterogeneity			0.04		0.08	
Baseline						
<1 serving/mo	95	73	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1 serving/mo to <1 serving/wk	175	175	0.80 (0.62, 1.04)	0.99 (0.75, 1.31)	0.83 (0.63, 1.08)	1.04 (0.77, 1.40)
1 serving/wk to <2 servings/wk	122	172	0.64 (0.49, 0.85)	1.16 (0.87, 1.54)	0.63 (0.47, 0.84)	1.13 (0.84, 1.52)
≥2 servings/wk	34	38	0.42 (0.28, 0.62)	0.57 (0.38, 0.87)	0.44 (0.29, 0.67)	0.55 (0.36, 0.84)
P-trend			<0.001	0.003	<0.001	0.001
P-heterogeneity			0.02		0.03	

[1]Results are from competing risks models. All of the models adjusted for age, calendar time, race (white, African American, Asian American, or other), height (≤68, >68–70, >70–72, or >72 inches), BMI at age 21 y (kg/m^2; <20, 20 to <22.5, 22.5 to <25, or ≥25), current BMI (<21, 21 to <23, 23 to <25, 25 to <27.5, 27.5 to <30, or ≥30), vigorous physical activity (quintiles of metabolic equivalent task hours/wk), smoking (never, former/quit >10 y ago, former/quit ≤10 y ago, or current), diabetes (yes or no), family history of prostate cancer in father or brother (yes or no), PSA testing (yes; no, lagged by one period to avoid counting diagnostic PSA tests as screening; cumulative average models only), use of multivitamins (yes or no), total calories (continuous), and intakes of calcium, α-linolenic acid, supplemental vitamin E, alcohol (quintiles), and coffee (none, <1, 1 to <2, 2 to <3, or ≥3 cups/d). ERG, v-ets avian erythroblastosis virus E26 oncogene homolog; HPFS, Health Professionals Follow-Up Study; PSA, prostate-specific antigen; Q, quintile; ref, reference; RP, radical prostatectomy.

[2]1 serving = 0.5 cup (118.294 mL) tomato sauce.

(61, 62). If tomato sauce promotes more antioxidation than lycopene, then the former might be more effective in reducing the DNA double-strand breaks that result in *TMPRSS2:ERG*. Additional studies should test the robustness of our findings.

It was also surprising that associations between exposures and prostate cancer overall were stronger for cases assayed for ERG than for all men with disease. Given the limited number of ERG-assayed cases, it seems likely that these differences were due to chance. It is also possible, however, that there are true differences between all men diagnosed with prostate cancer and those with tumors assayed for ERG. In our study, men in the latter group were more likely diagnosed earlier, and thus in the pre-PSA era. Ours and a previous study in the HPFS cohort (10) found that lycopene was more strongly associated with prostate cancer in the pre-PSA than in the PSA era. In addition, men treated with RP tended to be healthier than men treated otherwise, and lycopene could be more effective in protecting against prostate cancer in healthier men. For example, men treated with RP were more likely never smokers, and smoking likely decreases plasma lycopene (63), which could inhibit its protective effects. A final explanation stems from the upregulation of the insulin-like growth factor I (IGF-I) axis in prostate cancer (64). Lycopene inhibits IGF-I (13, 65–67), which tends toward higher concentrations in younger populations. The protective action of lycopene via the inhibition of IGF-I could thus be more impactful in younger men, such as the subset with disease treated with RP.

This mechanism would unlikely differ according to *TMPRSS2:ERG* subtype, and thus could explain inverse effects for ERG-negative disease.

Analyses of baseline exposures were more strongly inverse than analyses of cumulative average exposures. Lycopene could act early in the disease process, thereby rendering remote lycopene intake most etiologically relevant. *TMPRSS2:ERG* is thought to occur early in prostate carcinogenesis because it is not detected in benign prostate (68), is detected in ~20% of high-grade prostatic intraepithelial neoplasia (68–70), and is most often homogenously present or absent in any tumor nodule, even over time (71, 72). Earlier-life lycopene exposures might thus be more likely to affect the presence or absence of *TMPRSS2:ERG*. In our study, however, baseline exposures were more strongly associated with both ERG-positive and ERG-negative disease.

Several explanations have been proffered for the mixed evidence with regard to associations between lycopene, tomato products, and prostate cancer (9, 10). Another that may in part explain inconsistencies is that studies were conducted in populations with different prevalences of molecular subtypes. In populations in whom cancers develop with a lower frequency of a subtype associated with lycopene or tomato products, we might not see strong associations between exposure and disease risk overall. For example, Asian patients with prostate cancer have a lower prevalence of *TMPRSS2:ERG* (40). Studies with a high prevalence of Asian men have not found associations between lycopene and tomato products with prostate cancer (73–75). It should be noted, however, that studies in non-Asian populations have also returned null results (76–84).

There are limitations to our study that are worth noting. Restricting cases to men treated with RP rendered the entire population of men who did not develop prostate cancer an inappropriate comparison group. We instead needed a comparison group of men who would have received RP had they been diagnosed with disease. To address this potential bias, we implemented inverse probability weighting. In doing so, the interpretation of the results changed from risk of prostate cancer overall to risk of prostate cancer treated with RP with ERG status available. In any case, the results were similar regardless of weighting, and men with ERG status available were not especially different from other men with prostate cancer. We may not have completely accounted for the bias, but the weights accounted for the presumably most important clinical characteristics (stage, Gleason score, and PSA at diagnosis). An ideal next step would be to measure ERG status in biopsy tissue, which would be available for virtually all men with prostate cancer.

Another limitation is possible misclassification of diet. Given the prospective nature of the study, however, we would expect any bias in effect estimates that compared extreme quantiles to be toward the null. The evaluation of cumulative average intake also likely helped to minimize error. Observational research is limited by the possibility of uncontrolled confounding. In this case, it seems unlikely that it could entirely account for our results, given the strength of the associations and the similarity between age-adjusted and multivariable results. Our analyses of cumulative average intake did not entirely account for the possibility of time-varying confounding, but it likely was not a substantial issue given the inverse results of analyses of baseline intake.

To our knowledge, only one case-control study (7) and no other cohort studies have examined risk factors for differential associations with distinct molecular subtypes of prostate cancer. Few cancer epidemiology cohorts maintain prostate tissue repositories. The field also lacks a consensus with regard to the important molecular subtypes of prostate cancer. Our study is unique in its large prostate tumor tissue repository linked with long-term follow-up. Another strength is the richness of covariate data, which allowed us to account for potential confounders.

Our results suggest possible validity of the hypothesis that prostate cancers that are positive for *TMPRSS2:ERG* are etiologically distinct from cancers that are negative for the fusion. They also align with an ongoing paradigm shift in prostate cancer epidemiology. The continued study of prostate cancer as a single disease may conceal associations with particular subtypes. Future research should focus on identifying additional molecular subtypes that are important for differences in disease initiation and promotion, as well as the identification of risk factors by *TMPRSS2:ERG* status. Such research has the potential to improve opportunities for prevention of a disease for which there is little agreement on how to reduce risk.

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