Research Article

Cancer Prevention Research

A Functional Variant in *NKX3.1* Associated with Prostate Cancer Risk in the Selenium and Vitamin E Cancer Prevention Trial (SELECT)

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Abstract

NKX3.1 is an androgen-regulated prostate tumor suppressor protein. We previously found that antioxidant administration (*N*-acetylcysteine) in the *Nkx3*.1 knockout mouse model promoted prostate epithelial proliferation, suggesting that NKX3.1 activity modifies the effect of antioxidant administration on prostate carcinogenesis. Interestingly, administration of the antioxidant vitamin E significantly increased prostate cancer risk in the Selenium and Vitamin E Cancer Prevention Trial (SELECT), suggesting that our animal experiments may be relevant to humans. To determine whether NKX3.1 played a role in increased human prostate cancer risk associated with antioxidant administration in SELECT, we investigated the joint risk of antioxidant administration and *NKX3.1* genotypes previously found to be associated with decreased NKX3.1 mRNA expression (rs11781886) or DNA-binding activity *in vitro* (rs2228013) in the SELECT biomarker case–cohort substudy (1,866 cases; 3,135 non-cases). Multivariable COX regression models were developed to determine the joint association of *NKX3.1* genotypes with administration of vitamin E, selenium, or the combination, compared with placebo. The CC genotype at rs11781886 combined with selenium administration was associated with increased overall prostate cancer risk [HR, 1.676; 95% confidence interval (CI), 1.011–2.777; $P = 0.045$] and low-grade prostate cancer risk (HR, 1.811; 95% CI, 1.016–3.228; $P = 0.0441$). Similarly, the rs11781886 minor allele (CC+CT) combined with vitamin E administration was significantly associated with increased prostate cancer risk (HR, 1.450; 95% CI, 1.117–1.882; $P = 0.0052$). Our results indicate that variation in *NKX3.1* expression combined with selenium or vitamin E treatment modifies the risk of prostate cancer. Genetic background may modulate the effects of antioxidant supplementation thought to act as chemoprevention agents. *Cancer Prev Res; 7(9); 950–7. ©2014 AACR.*

Introduction

Secondary results from 2 previous clinical trials (1, 2) suggested that selenium or vitamin E supplementation could reduce prostate cancer incidence. The multicenter Selenium and Vitamin E Cancer Prevention Trial (SELECT) was initiated in 2001 to directly test the efficacy of these agents in preventing prostate cancer (3, 4). However, neither selenium nor vitamin E alone or in combination reduced the risk of prostate cancer; rather, all supplementation groups displayed elevated risk, with the 17% increased risk with vitamin E supplementation reaching statistical significance compared with placebo (5). Recent studies suggest that *NKX3.1* is a genetic risk factor for prostate cancer development that in animal models may also modulate the response to antioxidant supplementation (6–10). Nkx3.1 is a homeodomain containing haploinsufficient prostate tumor suppressor. Nkx3.1 directly regulates several enzymes that control oxidative stress levels [including glutathione peroxidase (Gpx2), peroxiredoxin 6 (Prdx6), and quiescin Q6 sulfhydryl oxidase 1 (Qsox1)], and *Nkx3.1*-mutant mice exhibit elevated oxidative stress levels in the prostate (9, 10). With advanced age, heterozygous and homozygous *Nkx3.1*-mutant mice display prostate lesions resembling prostatic intraepithelial neoplasia (PIN; refs. 11–13).

Elevated oxidative stress due to dysregulation of oxidant enzymes upon NKX3.1 loss has been proposed as a potential mechanism of prostate tumorigenesis in *NKX3.1*-deficient prostate cells (9, 14, 15). Antioxidant supplementation would thus be expected to reverse the oxidative stress caused by low NKX3.1 activity, thereby lowering the cancer risk. Surprisingly, however, supplementation of *Nkx3.1-null* mice with the antioxidant *N*-acetylcysteine

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doi: 10.1158/1940-6207.CAPR-14-0075

(NAC) increased, rather than decreased, prostate epithelial proliferation (10). These results are reminiscent of the SELECT trial, where vitamin E supplementation in cancer naïve individuals increased prostate cancer risk (5). Here we addressed a similar question in humans by determining whether 2 prostate cancer-related polymorphisms in *NKX3.1* (rs11781886 and rs2228013) modulate prostate cancer risk in men taking antioxidant supplementation in the SELECT trial. The minor C allele of the rs11781886 variant present in the 5′-untranslated region (UTR) of the *NKX3.1* gene leads to reduced *NKX3.1* mRNA expression by altering the binding of the transcription factor SP1 (16). The rs2228013 variant, on the other hand, codes for a variant NKX3.1 protein (R52C) with altered NKX3.1 phosphorylation and DNA-binding activity *in vitro* (17).

Materials and Methods

Study populations

SELECT was a randomized controlled clinical trial with 35,533 participants in the United States, Canada, and Puerto Rico. The study population characteristics have been described (5, 18). A smaller case–cohort population representative of the entire SELECT population was designed to use in biomarker studies and was the basis of this study. Men in the case–cohort population were stratified into 9 age/race cohorts: <55 (African American only), and 55–59, 60–64, 65–69, ≥70 years for both African Americans and others. Beginning in 2005 and annually until 2009, cases were matched to randomly selected men within each stratum; African American men were matched with a ratio of 1:3, whereas non–African American men were matched with a ratio of 1:1.5. (The oversampling of the African American strata was done to increase the precision of estimates in this higher risk group.) A man could be both a "case" and a randomly selected member of the subcohort. Cases and matching members of the subcohort had their samples pulled from the repository and processed (i.e., baseline plasma levels of nutrients measured, baseline toenail selenium measured, DNA extracted from buffy coat). A case–cohort was chosen over a case–control design because it allowed this annual processing. At the end of the intervention phase of SELECT, most samples had been processed and were ready for use, and matched annual selection reduced potential measurement errors due to analysis of aging samples. (The final case–cohort match was performed on July 31, 2009, and cases used in this analysis are as of July 31, 2009.) The subjects from the case–cohort population genotyped for this analysis included 1,866 cases and 3,135 non-cases for a total of 5,001 men.

NKX3.1 genotyping

Genotyping was performed using the ABI Prism TaqMan Allelic Discrimination Assays for rs11781886 and rs2228013. SNP rs11781886 is found in the 5′UTR of *NKX3.1* (Fig. 1) and leads to lower *NKX3.1* expression (16), whereas rs2228013 is found in the second exon of *NKX3.1* (Fig. 1) and alters NKX3.1 phosphorylation and DNA-binding activity *in vitro* (17). Single SNP allelic discrimination was carried out using the ABI 7900HT at the Dana Farber/Harvard Cancer Center High Throughput Genotyping Core Facility (Boston, MA). The call rate was 99.3% to 99.7%. The distributions of *NKX3.1* genotypes were similar across SELECT study arms; see Table 1.

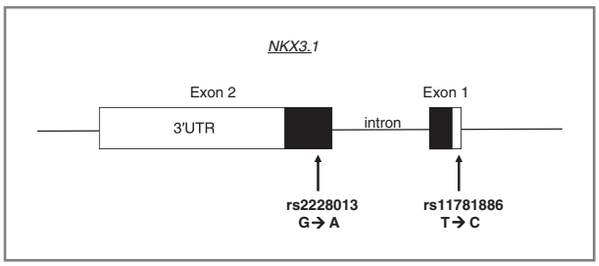

Figure 1. Location of *NKX3.1* SNPs genotyped in this study. Modified from the study by Akamatsu et al. (16), this image depicts the genetic location of SNP rs11781886 in the 5′UTR of the *NKX3.1* gene (16) and rs2228013 in the second exon of the *NKX3.1* gene (17).

Statistical analysis

Before the primary analysis, we considered the possibility that *NKX3.1* genotypes affect prostate cancer detection by evaluating Spearman correlations between PSA and *NKX3.1* genotype, with genotype coded 0, 1, or 2 for rs11781886 (counting the number of C alleles) and 0/1 for rs2228013 (absence or presence of A allele).

Prostate cancer outcomes included total prostate cancer, low-grade, and high-grade disease. HRs, associated confidence intervals (CI), and *P* values summarizing the association between *NKX3.1* genotype and prostate cancer risk were calculated using a Cox proportional hazards model. In addition, we hypothesized that *NKX3.1* genotypes might affect participants' response to study supplements, thus separate models included interactions between the SNPs and treatment assignment. Because the case–cohort was constructed as a stratified random sample across 9 age/race strata, the proportional hazards model was stratified by the 9 age/race strata with each strata weighted by the inverse of the subcohort selection probability. Covariates of interest defined *a priori* and included in final models were baseline family history of prostate cancer (yes or no), smoking status (nonsmoker, current smoker, former smoker), and body mass index (continuous). Because age and race were used as stratification factors, they were not used as covariates.

Cases outside the subcohort enter the proportional hazards model just before diagnosis and remain in until diagnosis. Non-cases in the subcohort enter the model at randomization and continue until they are censored (as of the earlier of July 31, 2009 or the date they were last known to be alive/date of death). Cases in the subcohort appear in the model twice: once treated as non-cases in the subcohort (entering at randomization, censored just before diagnosis) and once treated as cases outside the subcohort (19). In constructing the pseudo likelihood function, we chose the weighting method of Prentice for cases because it produced less biased estimates in a simulation study (20).

Table 1. Mean baseline PSA levels with genotype at rs11781886 in SELECT control cohort

	Intervention arm											
	Placebo			Vitamin E			Selenium			Vitamin E + selenium		
Genotype rs11781886	Mean adjusted PSA, ng/mL	SD	*n*	Mean adjusted PSA, ng/mL	SD	*n*	Mean adjusted PSA, ng/mL	SD	*n*	Mean adjusted PSA, ng/mL	SD	*n*
TT	1.32	0.93	424	1.31	0.96	478	1.35	0.90	488	1.30	0.87	434
CT	1.41	0.96	340	1.43	0.95	304	1.42	0.95	298	1.47	0.90	337
CC	1.46	0.97	61	1.65	1.07	72	1.54	0.97	50	1.38	0.85	49

Genotype effects for rs11781886 were calculated using a 3-level model (TT, CT, CC), unless otherwise noted. Genotype effects for rs2228013 were calculated using a 2-level model (GG, AG/AA) due to the low minor allele frequency. Target genotypes for rs11781886 were modeled in a joint effects model relative to the TT genotype in the placebo arm. Individual HRs were calculated for each of the 3 possible genotypes and 4 intervention arms. An additional analysis was done to test for linear trend, where the genotypes were modeled 0, 1, and 2 for TT, CT, and CC, respectively. Also, a joint effects model and linear trend analysis were performed using a 2-level model for these SNPs, with TT compared with CT and CC genotypes combined for rs11781886. The target SNP rs2228013 was modeled in a joint effects model relative to the GG genotype in the placebo arm. Individual HRs were calculated for the GG compared with AG and AA genotypes combined, due to the small number of samples with the AA genotype and the 4 intervention arms. An additional analysis was done to test for linear trend, where the genotypes were modeled 0 and 1 for GG and AG/AA, respectively. All statistical analyses were performed using SAS version 9.2 software (SAS Institute). All statistical tests are 2-sided, and $P < 0.05$ was considered statistically significant.

Results

Study population

Table 2 describes baseline study population characteristics in the case–cohort subpopulation within the SELECT trial. Of the 1,866 cases, 1,081 were considered low-grade (Gleason ≤ 6) and 540 high-grade (Gleason 7–10), whereas 245 had unknown grade. In general, the case–cohort participants reflected the characteristics of the overall SELECT population. One exception to this is race distribution, due to the planned overselection within the African American strata.

Association between baseline PSA and *NKX3.1* genotype

Baseline prostate-specific antigen (PSA) levels were higher among men who were diagnosed with prostate cancer at follow-up (Table 2). Genotype at rs2228013 was not associated with baseline PSA level (GG: $P = 0.3090$ and AG/AA: $P = 0.7852$). In contrast, the C allele of rs11781886 genotype was significantly associated with increasing baseline PSA ($P < 0.0001$). However, the numerical difference in PSA between genotypes was small (0.1–0.25 ng/mL) and unlikely to affect prostate cancer detection. Furthermore, the association between *NKX3.1* rs11781886 genotype and PSA was consistent among intervention arms (Table 1), suggesting that any effect of PSA would be carried across all study arms and thus is unlikely to bias our results of *NKX3.1* genetic effects between study arms. Thus, multivariate analysis for prostate cancer risk associated with all SNPs did not include baseline PSA level.

NKX3.1 genotype and prostate cancer risk in the SELECT sub-study

In analysis of all participants in the SELECT case–control cohort (all 4 intervention arms combined), the main effects of *NKX3.1* genotypes (additive model) were not significantly associated with total prostate cancer risk at follow-up (Table 3). Similarly, *NKX3.1* genotypes were not significantly associated with low-grade or high-grade prostate cancer risk.

Interaction between *NKX3.1* genotypes and antioxidant supplementation

In models containing interactions between the treatment arm and the *NKX3.1* genotype, the CC genotype at rs11781886 was significantly associated with an increased risk of total prostate cancer in the selenium arm (HR, 1.676; 95% CI, 1.011–2.777; $P = 0.045$). The CT genotype at rs11781886 in the vitamin E arm was also significantly associated with an increased risk of total prostate cancer (HR, 1.500; 95% CI, 1.124–1.971; $P = 0.0036$; Table 4). Having at least one C allele (i.e., CC or CT) was associated with a marginally significant increase in overall prostate cancer risk (HR, 1.277; 95% CI, 0.976–1.669; $P = 0.0744$) in the selenium arm, and a significant 45% increased overall prostate cancer risk (HR, 1.450; 95% CI, 1.117–1.882; $P = 0.0052$) in the vitamin E arm. These effects were not apparent in the vitamin E + selenium arm of the study. In contrast, analysis of rs2228013 showed no effect on prostate cancer risk in any of the intervention arms.

Table 2. Baseline characteristics of SELECT case–control cohort ($N = 5{,}001$)

Characteristic	Non-cases, *n* (%) $N = 3{,}135$	Cases, *n* (%) $N = 1{,}866$	High-grade cases, *n* (%) $N = 540$	Low-grade cases, *n* (%) $N = 1{,}081$
Age, y				
<55	126 (4.0%)	42 (2.3%)	11 (2.0%)	22 (2.0%)
55–59	832 (26.5%)	503 (27.0%)	123 (22.8%)	311 (28.8%)
60–64	926 (29.5%)	565 (30.3%)	150 (27.8%)	339 (31.4%)
65–69	724 (23.1%)	437 (23.4%)	138 (25.6%)	242 (22.4%)
≥70	527 (16.8%)	319 (17.1%)	118 (21.9%)	167 (15.4%)
Race				
White (non-Hispanic)	2,175 (69.4%)	1,521 (81.5%)	439 (81.3%)	899 (83.2%)
African American	756 (24.1%)	253 (13.6%)	79 (14.6%)	130 (12.0%)
Other	204 (6.5%)	92 (4.9%)	22 (4.1%)	52 (4.8%)
Body mass index, kg/m^2				
<25	606 (19.3%)	357 (19.1%)	98 (18.1%)	207 (19.1%)
25–<30	1,466 (46.8%)	950 (50.9%)	244 (45.2%)	583 (53.9%)
≥30	1,052 (33.6%)	556 (29.8%)	197 (36.5%)	289 (26.7%)
Unknown	11 (0.4%)	3 (0.2%)	1 (0.2%)	2 (0.2%)
Smoking status				
Never	1,292 (41.2%)	894 (47.9%)	263 (48.7%)	516 (47.7%)
Former	1,553 (49.5%)	868 (46.5%)	246 (45.6%)	500 (46.3%)
Current	267 (8.5%)	99 (5.3%)	28 (5.2%)	63 (5.8%)
Unknown	23 (0.7%)	5 (0.3%)	3 (0.6%)	2 (0.2%)
Baseline PSA				
0.00–0.99	1,391 (44.4%)	134 (7.2%)	33 (6.1%)	73 (6.8%)
1.00–1.99	1,070 (34.1%)	486 (26.0%)	140 (25.9%)	269 (24.9%)
2.00–2.99	444 (14.2%)	627 (33.6%)	196 (36.3%)	360 (33.3%)
≥3	230 (7.3%)	618 (33.1%)	171 (31.7%)	378 (35.0%)
Unknown	0 (0.0%)	1 (0.1%)	0 (0.0%)	1 (0.1%)
History of diabetes				
No	2,737 (87.3%)	1,733 (92.9%)	491 (90.9%)	1,018 (94.2%)
Yes	398 (12.7%)	133 (7.1%)	49 (9.1%)	63 (5.8%)
First-degree relative with prostate cancer				
None	2,626 (83.8%)	1,284 (68.8%)	384 (71.1%)	733 (67.8%)
≥1	507 (16.2%)	582 (31.2%)	156 (28.9%)	348 (32.2%)
Unknown	2 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
SELECT intervention assignment				
Vitamin E + selenium	772 (24.6%)	448 (24.0%)	138 (25.6%)	257 (23.8%)
Vitamin E alone	800 (25.5%)	518 (27.8%)	148 (27.4%)	291 (26.9%)
Selenium alone	782 (24.9%)	465 (24.9%)	138 (25.6%)	265 (24.5%)
Placebo	781 (24.9%)	435 (23.3%)	116 (21.5%)	268 (24.8%)
SNP: rs11781886				
TT	1,717 (54.8%)	984 (52.7%)	273 (50.6%)	579 (53.6%)
CT	1,184 (37.8%)	737 (39.5%)	227 (42.0%)	411 (38.0%)
CC	211 (6.7%)	136 (7.3%)	37 (6.9%)	86 (8.0%)
Unknown	23 (0.7%)	9 (0.5%)	3 (0.6%)	5 (0.5%)
SNP: rs2228013				
GG	2,864 (91.4%)	1,693 (90.7%)	488 (90.4%)	978 (90.5%)
AG	233 (7.4%)	150 (8.0%)	45 (8.3%)	90 (8.3%)
AA	5 (0.2%)	2 (0.1%)	0 (0.0%)	2 (0.2%)
Unknown	33 (1.1%)	21 (1.1%)	7 (1.3%)	11 (1.0%)

We next examined the interaction between *NKX3.1* genotypes and antioxidants in low- and high-grade prostate cancer. The CC genotype at rs11781886 was significantly associated with increased risk of low-grade prostate cancer in the selenium arm (HR, 1.811; 95% CI, 1.016–3.228; $P = 0.0441$; Table 5). Furthermore, the CT genotype at

Table 3. Effect of polymorphisms rs11781886 and rs2228013 on total, low-grade, and high-grade prostate cancer risk in all participants of SELECT case–control cohort

Polymorphism	HR (95% CIs)	*P*
Total prostate cancer		
rs11781886	1.072 (0.967–1.188)	0.1852
rs2228013	0.953 (0.759–1.196)	0.6773
Low grade		
rs11781886	1.076 (0.951–1.218)	0.2463
rs2228013	1.008 (0.771–1.318)	0.9529
High grade		
rs11781886	1.099 (0.939–1.286)	0.2390
rs2228013	0.933 (0.654–1.329)	0.6994

rs11781886 was associated with an increased risk of high-grade prostate cancer in the vitamin E arm (HR, 1.753; 95% CI, 1.146–2.680; $P = 0.0096$; Table 6), such that having at least one C allele at rs11781886 (genotype CT or CC) was associated with a significant 64% increase in high-grade prostate cancer risk (HR, 1.638; 95% CI, 1.089–2.463; $P = 0.0178$) with vitamin E supplementation.

Discussion

The search for an effective and nontoxic agent to prevent prostate cancer has been disappointing to date. The use of chemoprevention as an approach to reduce prostate cancer risk and mortality may rely upon combining information on personal genetic susceptibility with the potential benefits and toxicities of agents on individual patients (21). Using the SELECT biorepository, we investigated the relationship between 2 functional variants in the prostate tumor suppressor gene *NKX3.1* with overall prostate cancer risk and risk of low- and high-grade prostate cancer risk among men randomized to take vitamin E and/or selenium supplements.

Our analysis was prompted by our earlier observation that *Nkx3.1*-mutant mice showed increased, rather than decreased, prostate epithelial proliferation when an antioxidant supplement was administered (10). *Nkx3.1*-mutant mice exhibit dysregulation of genetic pathways responsible for regulation of ROS and elevated oxidative stress (10), but inhibition of ROS caused a protumorigenic phenotype, suggesting that a reactive oxygen species (ROS)-mediated inhibition of proliferation may be lost in these early lesions. Therefore, we hypothesized that *NKX3.1* genotype modulates prostate cancer risk upon antioxidant supplementation. Notably, in both the selenium and vitamin E arms, presence of the minor allele at rs11781886 was associated with a significantly increased risk of prostate cancer. Thus, in the setting of selenium or vitamin E supplementation, reduced NKX3.1 levels may permit proliferation of the prostate epithelial cells and increase the risk of prostate cancer. Although the mechanism by which this may occur in humans is not clear, this finding mirrors our results from treatment of *Nkx3.1* mutant mice with the antioxidant, NAC.

The reason that a significant increase in prostate cancer risk is observed with vitamin E supplementation in the CT genotype but not in the CC genotype at rs11781886 is presently unclear but may be related to the fact that the number of subjects with the CC genotype is substantially smaller than the TT or CT genotypes (Table 2), providing less statistical power to observe a significant interaction. It is also well established that *NKX3.1* is haploinsufficient, where loss of just one allele in humans or mice is associated with prostate tumor initiation (12). A number of molecular studies have identified discrete Nkx3.1 target genes that display dose-dependent regulation and may underlie the observed haploinsufficiency (22–24). A decrease in NKX3.1

Table 4. Effect of genotype at rs11781886 and rs2228013 on total prostate cancer risk in each intervention arm of the SELECT case–control cohort

	Intervention arm							
	Placebo *N* = 1,220		Vitamin E *N* = 1,318		Selenium *N* = 1,247		Vitamin E + selenium *N* = 1,216	
Genotype *rs11781886*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
All genotypes	1.000 (Ref)	Ref	1.178 (0.987–1.405)	0.0688	1.091 (0.913–1.304)	0.3367	1.021 (0.853–1.222)	0.8171
TT	1.000 (Ref)	Ref	1.142 (0.891–1.463)	0.2951	1.074 (0.836–1.379)	0.5753	1.218 (0.947–1.567)	0.1238
CT	1.175 (0.895–1.542)	0.2448	1.500 (1.124–1.971)	0.0036	1.218 (0.918–1.617)	0.1710	0.966 (0.733–1.272)	0.8048
CC	1.144 (0.690–1.898)	0.6015	1.233 (0.744–2.042)	0.4162	1.676 (1.011–2.777)	0.0450	0.987 (0.541–1.803)	0.9671
P_{trend}	0.2897		0.8548		0.6457		0.0634	
rs2228013								
GG	1.000 (Ref)	Ref	1.165 (0.968–1.403)	0.1066	1.064 (0.882–1.283)	0.5191	1.037 (0.859–1.252)	0.7074
AG or AA	0.912 (0.574–1.447)	0.6952	1.137 (0.742–1.740)	0.5557	1.365 (0.876–2.128)	0.1685	0.740 (0.456–1.203)	0.2247
P_{trend}	0.6952		0.8330		0.2934		0.4722	

Table 5. Effect of genotype at rs11781866 and rs2228013 on low-grade prostate cancer risk in each arm of the SELECT case–control cohort

	Intervention arm							
	Placebo		Vitamin E		Selenium		Vitamin E + selenium	
Genotype Low-grade cases	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
All genotypes	1.00 (ref)	Ref	1.070 (0.867–1.319)	0.5294	1.004 (0.812–1.241)	0.9728	0.932 (0.752–1.154)	0.5166
rs11781886								
TT	1.000 (ref)	Ref	1.159 (0.864–1.554)	0.3251	1.018 (0.754–1.375)	0.9054	1.182 (0.874–1.599)	0.2764
CT	1.261 (0.917–1.734)	0.1537	1.267 (0.910–1.765)	0.1608	1.172 (0.836–1.643)	0.3560	0.915 (0.658–1.273)	0.5977
CC	1.160 (0.649–2.074)	0.6156	1.341 (0.742–2.424)	0.3312	1.811 (1.016–3.228)	0.0441	1.010 (0.487–2.095)	0.9795
P_{trend}	0.2212		0.7016		0.6488		0.0802	
rs2228013								
GG	1.000 (ref)	Ref	1.079 (0.865–1.346)	0.4997	0.987 (0.789–1.235)	0.9082	0.956 (0.764–1.197)	0.6968
AG or AA	0.974 (0.568–1.670)	0.9232	1.036 (0.621–1.728)	0.8915	1.386 (0.833–2.308)	0.2091	0.761 (0.431–1.343)	0.3461
P_{trend}	0.9232		0.9705		0.3317		0.6115	

expression resulting from one C allele may be sufficient to allow for a promotion of prostate cancer upon vitamin E supplementation. Importantly, our previous studies with NAC supplementation in *Nkx3.1*-mutant mice were performed only in $Nkx3.1^{-/-}$ and $Nkx3.1^{+/+}$ mice but not $Nkx3.1^{+/-}$ mice, and it is possible that $Nkx3.1^{+/-}$ mice may have shown the same or greater increase in proliferation upon NAC supplementation.

Our findings indicate that the genetically heterogeneous nature of the subjects in SELECT may have masked significant biologic effects of antioxidant supplementation in subsets of participants. In this regard, the previously reported significantly increased risk of prostate cancer with vitamin E supplementation in SELECT (5) may be partially due to a substantial increase in risk among those SELECT participants with low *NKX3.1* expression in the vitamin E supplementation group. In addition, we suggest that those with the *NKX3.1* rs11781886 minor allele contributed to the overall nonstatistically significant elevation in prostate cancer risk observed in the selenium and vitamin E + selenium arms.

Unlike Gelmann and colleagues (17), we found no elevation in high-grade prostate cancer risk due to rs2228013 in the SELECT case–cohort, nor did rs2228013 affect total or low-grade risk in the case–cohort overall or in any intervention arm. rs2228013 has been shown to modulate NKX3.1 function *in vitro* (17); however, unlike rs11781886 (16), *in vivo* and human tissue studies to

Table 6. Effect of genotype at rs11781866 and rs2228013 on high-grade prostate cancer risk in each arm of the SELECT case–control cohort

	Intervention arm							
	Placebo		Vitamin E		Selenium		Vitamin E + selenium	
Genotype High-grade cases	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
All genotypes	1.00 (Ref)	Ref	1.250 (0.942–1.658)	0.1224	1.201 (0.902–1.598)	0.2099	1.212 (0.911–1.612)	0.1868
rs11781886								
TT	1.000 (Ref)	Ref	1.015 (0.672–1.533)	0.9447	1.059 (0.710–1.579)	0.7796	1.297 (0.871–1.931)	0.2002
CT	1.027 (0.656–1.609)	0.9058	1.753 (1.146–2.680)	0.0096	1.308 (0.839–2.039)	0.2355	1.078 (0.692–1.678)	0.7401
CC	1.164 (0.519–2.611)	0.7123	1.131 (0.515–2.484)	0.7600	1.325 (0.559–3.140)	0.5224	1.079 (0.435–2.676)	0.8695
P_{trend}	0.7586		0.3596		0.6433		0.4247	
rs2228013								
GG	1.000 (Ref)	Ref	1.209 (0.896–1.630)	0.2138	1.173 (0.869–1.584)	0.2969	1.197 (0.887–1.616)	0.2407
AG or AA	0.909 (0.444–1.862)	0.7938	1.223 (0.635–2.354)	0.5472	1.240 (0.593–2.589)	0.5677	0.928 (0.448–1.922)	0.8413
P_{trend}	0.7938		0.8278		0.7724		0.7592	

analyze the effect of SNP on NKX3.1 expression or activity in the human prostate have not been reported.

At present, the precise biologic mechanisms behind the increased risk of prostate cancer due to rs11781886 and antioxidant supplementation are unknown and will require further investigation. Prostates of *Nkx3.1*-deficient mice treated with NAC showed increased expression of gene sets involved in positive regulation of cell proliferation and chemokine/growth factor signaling (10). Further studies will be needed to dissect how manipulation of ROS levels through micronutrient or antioxidant administration in *Nkx3.1*-deficient cells affects proproliferation gene expression. Nevertheless, the possibility also remains that the effects seen in both the *Nkx3.1* mouse model and in the SELECT subjects might be related to non-antioxidant functions of the agents used (NAC, vitamin E, and selenium).

It is unclear why there was no evidence of effect modification by rs11781886 in the study arm combining vitamin E and selenium supplementation. The complex nature of the relationship between baseline selenium levels and selenium and vitamin E supplementation in prostate cancer risk is highlighted in a recent case–cohort study analyzing SELECT (25). Kristal and colleagues showed that unlike a previous report (2), selenium supplementation did not decrease risk of prostate cancer in men with low selenium status and instead increased the risk of prostate cancer in men with high selenium status (25). However, vitamin E supplementation increased prostate cancer risk only in those with low baseline selenium levels (25). Thus, the lack of a statistically significant modification of risk by rs11781886 in the vitamin E + selenium arm, may be due to competing effects of the 2 antioxidants when administered together. Indeed, not only individual genetic variation but also levels of other antioxidants such as selenium may influence the risk of developing prostate cancer with supplementation.

Past studies have reported on the association of prostate cancer risk with genetic variants in antioxidant genes, including superoxide dismutase 1 (*SOD1*), manganese superoxide dismutase (*SOD2*), and the DNA repair enzymes *hOGG1* and *XRCC1* (26–30). These and other variants could affect the outcome of antioxidant supplementation on prostate cancer risk.

The strengths of our study include the large sample size from randomized trial population with systematic data collection afforded by SELECT as well as the evaluation of PSA before and during the trial. Randomization of participants to each study arm greatly reduces the potential for bias associated with selective use and administration of nutritional supplements. Furthermore, vitamin E and selenium supplement doses and regiments were standardized, reducing variation in supplement exposure within each study arm. The serum levels of the supplements support good adherence to each supplement protocol (18).

Limitations of the study include the inability to evaluate race-specific associations due the substudy structure and sampling, fewer high-grade cases for analysis, and a lower-than-ideal prevalence of the minor allele at rs11781886 and rs2228013 for a complete gene–dose or gene–gene interaction analysis. While no effect of selenium and/or vitamin E supplementation on prostate or lung cancer risk was observed in smokers in SELECT (data not shown), the number of smokers was relatively small (8% current smokers), which prevents SELECT from being an ideal study to assess smoker-specific prostate cancer risk. While chance is always an alternative explanation for findings such as ours, our analysis was based on an *a priori* hypothesis developed through our basic research. Replication in an independent population taking similar antioxidant supplements and at risk for prostate cancer will be necessary.

In conclusion, our results suggest that an individual's prostate cancer risk associated with selenium or vitamin E supplementation may be modified by *NKX3.1* genotype, in particular the rs11781886 C allele previously found to decrease NKX3.1 expression. These findings open a new avenue to explore the molecular events associated with *NKX3.1* polymorphisms and prostate tumorigenesis and reveal the significant impact that these and other gene–environment interactions may have on prostate cancer development. Such information could be used in the context of personalized medicine to identify those men most likely to benefit from antioxidant supplementation for cancer prevention or adjuvant care.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Tangen, E.A. Klein

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.E. Martinez, A.K. Darke, C.M. Tangen, P.J. Goodman, J.H. Fowke

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Acknowledgments

The authors thank SWOG investigators for providing access to the SELECT repository and to Dr. Lorelei Mucci (Dana Farber Cancer Institute) for facilitating genotyping studies.

Grant Support

This work was supported by the National Center for Research Resources, Grant UL1 RR024975-01, and is now at the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06; by the NIH grant RO1CA94858 (to S.A. Abdulkadir) and RO1CA121060 (to J.H. Fowke); by Public Health Service Cooperative Agreement Grant CA37429 awarded by the National Cancer Institute, NIH, Department of Health and Human Services, by the National Center for Complementary and Alternative 1 Medicine (NIH), and by developmental funds awarded to SWOG Cancer Cooperative Group Grant U10 CA32102; by the National Center for Research Resources, Grant UL1 RR024975-01, and is now at the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06; and by the Prostate Cancer Foundation (E.A. Klein).

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Received February 27, 2014; revised May 19, 2014; accepted May 25, 2014; published OnlineFirst June 3, 2014.

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