Research Article

Common Genetic Variation of the Calcium-Sensing Receptor and Lethal Prostate Cancer Risk

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Abstract

Background: Bony metastases cause substantial morbidity and mortality from prostate cancer (PCa). The calcium-sensing receptor (CaSR) is expressed on prostate tumors and may participate in bone metastases development. We assessed whether (i) common genetic variation in CaSR was associated with PCa risk and (ii) these associations varied by calcium intake or plasma 25-hydroxyvitamin D [25(OH)D] levels.

Methods: We included 1,193 PCa cases and 1,244 controls nested in the prospective Health Professionals Follow-up Study (1993–2004). We genotyped 18 CaSR single-nucleotide polymorphism (SNPs) to capture common variation. The main outcome was risk of lethal PCa (n = 113); secondary outcomes were overall (n = 1,193) and high-grade PCa (n = 225). We used the kernel machine approach to conduct a gene-level multi-marker analysis and unconditional logistic regression to compute per-allele ORs and 95% confidence intervals (CI) for individual SNPs.

Results: The joint association of SNPs in CaSR was significant for lethal PCa (P = 0.04); this association was stronger in those with low 25(OH)D (P = 0.009). No individual SNPs were associated after considering multiple testing; three SNPs were nominally associated (P < 0.05) with lethal PCa with ORs (95% CI) of 0.65(0.42–0.99): rs6438705; 0.65(0.47–0.89): rs13083990; and 1.55(1.09–2.20): rs2270916. The three nonsynonymous SNPs (rs1801725, rs1042636, and rs1801726) were not significantly associated; however, the association for rs1801725 was stronger in men with low 25(OH)D [OR(95%CI): 0.54(0.31–0.95)]. There were no significant associations with overall or high-grade PCa.

Conclusions: Our findings indicate that CaSR may be involved in PCa progression.

Impact: Further studies investigating potential mechanisms for CaSR and PCa, including bone remodeling and metastases are warranted.

Introduction

When prostate cancer metastasizes, it shows a high propensity to metastasize to bone, especially to areas of high bone turnover (1, 2). Bone metastases are difficult to treat and are a major cause of morbidity and mortality for patients with advanced prostate cancer, often causing severe pain, fractures, and death (1). Calcium homeostasis and bone remodeling are hypothesized to be important factors in the development of bony metastases in prostate cancer. Some epidemiologic studies have indicated that high intake of dietary calcium (3) and high serum calcium (4) may be associated with an increased risk of prostate cancer, particularly advanced or lethal disease. Moreover, increased parathyroid hormone (PTH) levels resulting in bone turnover promotes prostate bony metastases in animal studies (5). Vitamin D deficiency promotes prostate cancer growth in bone in animal studies (6) and epidemiologic studies have shown that men with the lowest plasma 25-hydroxyvitamin D [25(OH)D] levels are at the greatest risk for lethal (metastatic or fatal) prostate cancer (7–9).

A key regulator of calcium homeostasis is the calcium-sensing receptor (CaSR), a transmembrane receptor that controls PTH levels through expression in the parathyroid gland. Initially, CaSR was described in tissue with clear roles in calcium homeostasis such as bone, parathyroid, and kidney. However, more widespread expression of CaSR has been discovered in other tissues, including prostate, breast, and colon cancer (10–14) and CaSR may also influence several tumor-related processes independent of circulating calcium level regulation. For example, in prostate and breast cancer cells CaSR has proliferative and antiapoptotic effects, and may facilitate cell migration and bone metastases (15, 16). In addition, CaSR expression has...
been shown to be upregulated through a vitamin D response element in the gene’s promoter region (17).

Genetic variants in CaSR may underlie some of the variability in its function. The only epidemiologic study, conducted in African American men, found that a nonsynonymous single-nucleotide polymorphism (SNP; Q1011E—RS1801726) common in the African American population was associated with advanced, but not overall prostate cancer (18). Using prospectively collected data from the Health Professionals Follow-Up Study, we assessed whether genetic variation across CaSR was specifically associated with risk of lethal (distant metastatic or fatal) prostate cancer, given the biologic plausibility of its role in promoting metastatic disease. Moreover, we leveraged existing dietary and circulating biomarker data to assess whether these associations were modified by dietary and supplemental calcium intake or plasma 25(OH)D levels.

**Materials and Methods**

**Study population**

The Health Professionals Follow-up Study (HPFS) began in 1986 when 51,529 male health professionals aged 40 to 75 responded to a mailed questionnaire about their demographics, lifestyle, diet, and medical history (19). Medical and lifestyle information for this ongoing prospective cohort study was updated every 2 years via follow-up questionnaires; diet was updated every 4 years. The overall follow-up was over 94% and mortality assessment was greater than 98%. The current study was nested among the 18,018 (35%) of men who provided a blood specimen between 1993 and 1995. Specimens were processed within 24 hours of blood draw and plasma, erythrocytes and buffy coats were separated, aliquoted, and stored in liquid nitrogen freezers (20).

Incident cases of prostate cancer (diagnosed 1993–2004) were identified through the self-reported questionnaires or death certificates. Medical records and pathology reports were obtained from the majority (95%) of participants to confirm the diagnoses, and to abstract information on stage of disease (classified using the tumor, node, and metastasis system) and Gleason score (sum of major and minor components, 2–10). We used the pathologic stage and grade if available; otherwise we used clinical stage and grade. The cases were followed prospectively for mortality and metastases through March 2011. Deaths were identified via next-of-kin reports, mailings, and searches of the National Death Index, and confirmed by an Endpoints Committee through review of death certificates and medical records (90% of deaths and nearly all prostate cancer deaths were confirmed via medical record review). Medical record reviews were conducted blinded to exposure information. In addition, information on treatment and disease progression (e.g., metastases) was collected on all reported cases.

Men diagnosed with other types of cancer (except nonmelanoma skin cancer) before blood draw were excluded from the study. We defined the main outcome of lethal prostate cancer as having distant metastases at diagnosis or development of distant metastases during follow-up, or prostate cancer specific death during follow-up through March 2011. We also assessed overall cancer, tumors characterized as advanced stage at diagnosis (T3b/T4, M1/N1(T1-T4) or lethal, and tumors characterized as high Gleason grade, defined as a Gleason pattern of 4 + 3 to 10. For the analysis, we also excluded men diagnosed with stage T1a microscopic focal prostate cancer because these tumors are generally indolent and are most susceptible to detection bias because of differential rates of undergoing surgery for benign prostatic hyperplasia.

We used a risk set sampling strategy to select eligible controls; controls were free of known prostate cancer at the time the case was diagnosed and reported having a prostate-specific antigen (PSA) test after the date of blood draw to ensure a more equal opportunity for prostate cancer detection as the majority of cases had received a PSA test for screening or as part of their diagnostic evaluation). We randomly selected 1 eligible control per case and matched on age (exact year of birth), PSA screening history before blood draw, and season, year, and time of day of blood draw.

**SNP selection and genotyping**

Using the Tagger algorithm (21) implemented in the HaploView program (22) and dense genotyping data from the International HapMap Phase III CEU samples, we identified 18 linkage disequilibrium tagging SNPs to capture variation with R² > 0.8 within the CaSR gene with additional coverage of the areas 20 kb upstream and 10 kb downstream of the actual gene. We forced in 3 nonsynonymous SNPs from exon 7 with potential functionality (rs1801725, rs1042636, and rs1801726), otherwise selection was restricted to those with a minor allele frequency (MAF) of more than 5% in the reference panel.

All blood samples were identified with a research ID number only and matched case–control pairs were handled identically and assayed in the same batch in a blinded fashion. DNA was extracted from whole blood using a standard QIAmp kit (QIAGEN Inc.) protocol and genotyping was conducted using the Bio-trove OpenArray SNP Genotyping Platform at the Harvard Medical School-Partners Healthcare Center for Genetics and Genomics. Of the 18 genotyped SNPs, 17 had at least 95% genotype completion and 1 (rs7630625) had 92% completion. Multiple random blinded duplicate quality control samples were included and the concordance was 99.7%. If participants were missing genotype information, we imputed the values using the HapMap Phase III CEU data and the MACH imputation program (23).

**Calcium intake and plasma 25(OH)D**

Dietary and supplement calcium intake were assessed through a validated semiquantitative food-frequency questionnaire collected every 4 years (19). We used...
energy-adjusted cumulative average intake until the date
closest to, but before diagnosis to represent longer term
calium exposure. Because the relationship of calcium
and fatal prostate cancer has been shown to be
limited to the highest categories of calcium intake in our
cohort (3), we used the top tertile of total calcium intake as
the cut point for high calcium intake.

Plasma 25(OH)D was measured using radioimmuno-
sorbant assay (Dr. Bruce Hollis; ref. 24) in 4 separate
batches with mean intrapair coefficients of variation from
blinded quality control samples (5.4%, 5.6%, 14.8%, and
5.6%, for batches 1–4). Repeated blood samples taken
a mean of 3 years apart on a subsample of individuals (n =
144) yielded a Pearson correlation coefficient of 0.7
(adjusted for age, race, and season; ref. 25). We dichoto-
mized plasma 25(OH)D at the median value using batch
and season specific cut points and also created a batch and
season-standardized continuous measure of 25(OH)D.

All participants had both CaSR genotype and calcium
information and 99% of participants had both CaSR geno-
type and plasma 25(OH)D information available.

Analysis

To reduce the potential for population stratification we
restricted our analyses to Caucasian men of European
descent (95% of the HPFS Blood cohort). Hardy–Weinberg
equilibrium (HWE) tests for each of the SNPs was con-
ducted among controls using the Pearson’s goodness-of-
fit test using a cutoff of P < 0.001. One genotyped SNP
(rs1979869) had evidence for departure from HWE among
controls (P < 0.001), so we removed the genotyped values
for the SNP and instead used imputed values for this SNP.

To assess the global multimarker association across the
18 SNPs in CaSR, we used a logistic kernel-machine model
with a linear kernel function. The methodology has sev-
eral advantages, including the ability to capture the joint
effect of several SNPs in a gene, which may provide more
power to detect an overall association, as individual effect
estimates for each SNP are likely to be modest. Also, in
contrast to methods that create a single genetic score from
a set of proposed risk alleles, a benefit of the kernel
machine is that it does not require a priori knowledge of
directionality for the variants. In brief, the logistic kernel
machine model treats each of the included SNPs as a
random effect; desired covariates can be entered as fixed
effects. The null hypothesis that the variance of the SNP
random effects is 0 (i.e., that the SNPs individually or
jointly are not associated with disease) can be tested using
a score test. The kernel machine is computationally effi-
cient compared with other methods that require permu-
tation. The in-depth mathematical details of the logistic
kernel machine model have been described elsewhere
(26, 27).

Adjusted per-allele ORs and 95% confidence intervals
(CI) of each individual SNP and lethal prostate cancer
were assessed using unconditional logistic regression,
adjusting for age at blood draw. We reported the nominal
2-sided P values for these analyses without adjusting for
multiple testing; however, we also calculated a correction
for multiple comparisons using a method (28) that takes
into account the linkage disequilibrium within the set of
SNPs being tested to calculate the effective number of
independent tests. The 18 SNPs corresponded to 17 inde-
dependent tests; consequently a
P
value significance thresh-
old of 0.003 controls the experiment-wide Type I error rate
at the 0.05 level. We conducted stratified analyses to assess
whether the association of any of the CaSR SNPs varied by
high or low total calcium intake or plasma 25(OH)D levels
by the cut points defined previously. We also calculated
Wald P values for interaction by creating a multiplicative
interaction term for each SNP and the binary cut point for
total calcium intake (top tertile vs. bottom 2 tertiles) and a
continuous measure of 25(OH)D and entering it into the
model in addition to the main effects. Because the rela-
tionship between 25(OH)D and lethal prostate cancer has

Table 1. Characteristics of prostate cancer cases in the Health Professionals Follow-up Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All cases (n = 1193)</th>
<th>Lethal cases (n = 113)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at blood draw, y; mean (SD)</td>
<td>64.4 (7.8)</td>
<td>68.2 (7.9)</td>
</tr>
<tr>
<td>Age at diagnosis, y; mean (SD)</td>
<td>69.5 (7.5)</td>
<td>72.0 (7.8)</td>
</tr>
<tr>
<td>Stage, n, (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1, T2 (N0, M0)</td>
<td>958 (86)</td>
<td>62 (62)</td>
</tr>
<tr>
<td>T3a (N0, M0)</td>
<td>84 (8)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>T3b (N0, M0)</td>
<td>41 (4)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>T4 (N0, M0)</td>
<td>1 (0)</td>
<td>0</td>
</tr>
<tr>
<td>N1</td>
<td>14 (1)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>M1</td>
<td>15 (1)</td>
<td>15 (15)</td>
</tr>
<tr>
<td>Missing (n = 80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason grade, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>618 (56)</td>
<td>28 (30)</td>
</tr>
<tr>
<td>7: 3 + 4 or no major score defined</td>
<td>259 (24)</td>
<td>19 (21)</td>
</tr>
<tr>
<td>7: 4 + 3</td>
<td>106 (10)</td>
<td>12 (13)</td>
</tr>
<tr>
<td>8 to 10</td>
<td>119 (11)</td>
<td>33 (36)</td>
</tr>
<tr>
<td>Missing (n = 91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deaths/metastases because of prostate cancer, n (%)</td>
<td>113 (9)</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer deaths</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Bone or organ metastases on follow-up (alive)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Distant metastases at diagnosis (alive)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Time to diagnosis from blood draw, y; median (IQR)</td>
<td>5.2 (2.8, 7.6)</td>
<td>3.7 (2.0, 5.7)</td>
</tr>
<tr>
<td>Time to lethal prostate cancer (death/metastases) from diagnosis, y; median (IQR)</td>
<td>—</td>
<td>5.5 (3.1, 8.6)</td>
</tr>
</tbody>
</table>
been shown to be relatively linear (9), we used the continuous measure to improve power in the test for interaction. Analyses were conducted using SAS v9.2 (SAS Institute) and R (29) statistical packages. The Human Subjects Committee at the Harvard School of Public Health approved this study.

Results

A total of 1,193 incident prostate cancer cases and 1,244 controls were included in this study; 113 of the cases were characterized as lethal, 158 were advanced stage or lethal, and 225 were Gleason grade 4+3 or higher (Table 1).

Table 2 presents the global test for association between common genetic variation in CaSR and the per-allele ORs for each of the 18 SNPs and the main outcome lethal prostate cancer. Results from the logistic kernel machine including all 18 SNPs supported a significant association of common variation in CaSR and lethal prostate cancer ($P = 0.04$). In addition, this association across the gene with lethal prostate cancer was stronger among those with lower plasma 25(OH)D ($P = 0.007$) compared with those with high plasma 25(OH)D ($P = 0.56$) and lower total calcium intake ($P = 0.05$) compared with those with high total calcium intake ($P = 0.41$; Tables 3 and 4).

Considering the SNPs individually, 3 SNPs (rs6438705, rs7630625, and rs1801725) were nominally associated ($P < 0.05$) with risk of lethal prostate cancer, but did not remain significant after considering multiple testing (Table 2). Although none of the 3 nonsynonymous SNPs (rs1801725, rs1042636, and rs1801726) were significantly associated with lethal prostate cancer in the main effects analysis, rs1801725, the nonsynonymous SNP with the most common MAF in Caucasian populations, had an OR, 0.70; 95% CI, 0.46–1.06; $P = 0.09$. Moreover, the association for rs1801725 and lethal prostate cancer was suggestively stronger for those with low plasma 25(OH)D ($P_{interaction} = 0.14$; Table 3). Tables 3 and 4 describe the individual SNP results with lethal prostate cancer by high and low plasma 25(OH)D and calcium intake. In particular, the relation between rs2270916 and risk of lethal prostate cancer appeared to be confined to men with low plasma 25(OH)D ($P_{interaction} = 0.02$; Table 3). Finally, although the main effect for rs7637874 and lethal prostate cancer was not statistically significant there was suggestive effect modification by total calcium intake ($P_{interaction} = 0.008$; Table 4).

Overall, results were consistent when we added advanced stage cancers [T3b/T4, M1/N1(T1-T4)] to the lethal cases (Table 5). We found no significant associations for genetic variation in CaSR and risk of prostate cancer overall or high-grade disease (Table 5). Sensitivity analyses conducted for the individual SNP results were consistent when we excluded individuals with missing genotypes (data not shown).

### Table 2. Global CaSR association and individual SNP associations (per-allele OR and 95% CI) for lethal prostate cancer

<table>
<thead>
<tr>
<th>SNP name (minor, major allele)</th>
<th>Gene location</th>
<th>Minor allele frequency (controls)</th>
<th>OR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9820206(A,G)</td>
<td>Intron</td>
<td>0.25</td>
<td>0.86 (0.61–1.20)</td>
<td>0.38</td>
</tr>
<tr>
<td>rs6438705(A,G)</td>
<td>Intron</td>
<td>0.18</td>
<td>0.65 (0.42–0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>rs13083990(C,T)</td>
<td>Downstream</td>
<td>0.36</td>
<td>0.65 (0.47–0.89)</td>
<td>0.008</td>
</tr>
<tr>
<td>rs4678173(A,C)</td>
<td>Intron</td>
<td>0.34</td>
<td>1.21 (0.92–1.60)</td>
<td>0.17</td>
</tr>
<tr>
<td>rs1979869(C,T)</td>
<td>Intron</td>
<td>0.31</td>
<td>0.73 (0.51–1.03)</td>
<td>0.08</td>
</tr>
<tr>
<td>rs2270916(C,T)</td>
<td>Intron</td>
<td>0.14</td>
<td>1.55 (1.09–2.20)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs16832787(A,G)</td>
<td>Intron</td>
<td>0.24</td>
<td>1.21 (0.87–1.69)</td>
<td>0.26</td>
</tr>
<tr>
<td>rs2134223(A,G)</td>
<td>Intron</td>
<td>0.15</td>
<td>0.97 (0.66–1.44)</td>
<td>0.89</td>
</tr>
<tr>
<td>rs4639101(G,T)</td>
<td>Downstream</td>
<td>0.14</td>
<td>0.94 (0.62–1.42)</td>
<td>0.76</td>
</tr>
<tr>
<td>rs1501900(A,T)</td>
<td>Intron</td>
<td>0.21</td>
<td>1.15 (0.83–1.61)</td>
<td>0.39</td>
</tr>
<tr>
<td>rs1393198(C,T)</td>
<td>Intron</td>
<td>0.13</td>
<td>0.94 (0.62–1.44)</td>
<td>0.79</td>
</tr>
<tr>
<td>rs7612328(A,G)</td>
<td>Intron</td>
<td>0.50</td>
<td>1.10 (0.83–1.46)</td>
<td>0.50</td>
</tr>
<tr>
<td>rs7637874(C,T)</td>
<td>Intron</td>
<td>0.25</td>
<td>1.21 (0.88–1.65)</td>
<td>0.24</td>
</tr>
<tr>
<td>rs7840414(C,T)</td>
<td>Promoter</td>
<td>0.28</td>
<td>0.94 (0.69–1.29)</td>
<td>0.70</td>
</tr>
<tr>
<td>rs7606255(A,G)</td>
<td>Upstream</td>
<td>0.39</td>
<td>1.00 (0.74–1.34)</td>
<td>1.00</td>
</tr>
<tr>
<td>rs1801725(G,T)</td>
<td>Exon</td>
<td>0.16</td>
<td>0.70 (0.46–1.06)</td>
<td>0.09</td>
</tr>
<tr>
<td>rs1042636(A,G)</td>
<td>Exon</td>
<td>0.08</td>
<td>0.78 (0.45–1.34)</td>
<td>0.37</td>
</tr>
<tr>
<td>rs1801726(C,G)</td>
<td>Exon</td>
<td>0.04</td>
<td>1.41 (0.78–2.54)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*113 cases, 1,244 controls
Discussion

To our knowledge, our study is the first epidemiologic study to specifically assess genetic variation across CaSR and lethal prostate cancer risk in Caucasian men. Using a global multimarker test, we observed a statistically significant joint association for variation across CaSR with lethal prostate cancer; this association was stronger in those with lower plasma 25(OH)D. We observed that 3 common variants in the CaSR gene were nominally associated with the lethal prostate cancer risk. In addition, there was suggestive evidence that some of the individual associations varied by plasma 25(OH)D and total calcium intake. We did not observe an association with variation in CaSR and overall prostate cancer or high-grade prostate cancer.

Prostate cancer cells preferentially metastasize to bone, especially bone with higher remodeling activity (1). Calcium released during bone remodeling may stimulate a chemotactic effect to promote migration of cancer cells to bone via CaSR (30). In addition, activation of CaSR in tumor cells induces production of parathyroid hormone-related protein and leads to bone resorption and further calcium release, which could create an environment favorable to bony metastases (2, 31). CaSR activation has also been shown to have other oncogenic properties such as increased proliferation and decreased apoptosis.

Genome-wide association studies (GWAS) of the CaSR and serum calcium have found that the variant allele of a nonsynonymous SNP (rs1801725) and a SNP in high LD with rs1801725 (rs17251221) were associated with increased serum calcium (32, 33) magnesium, PTH, and decreased serum phosphate (33) and that these changes were consistent with a loss of function variant. This finding is intriguing in light of the suggestive protective association for those carrying the variant allele of rs1801725 and lethal prostate cancer observed in our data. This association was stronger in those with low 25(OH)D levels. As CaSR activation may lead to cancer progression, a variant resulting in a less active receptor is consistent with the observed protective association. Both calcium and vitamin D are important factors in calcium homeostasis and bone remodeling and low vitamin D may be a risk factor for lethal prostate cancer (7). In addition, the CaSR gene contains a vitamin D response element that has been shown to upregulate its expression in some tissues (17). Prior studies have found that high calcium intake (3) and low plasma 25(OH)D levels (9) are associated with lethal prostate cancer and the evidence that prostate cancers metastasize favor bone with high remodeling activity (1) provide a basis for these relationships; however, the mechanisms for these associations are still unclear. Our observations that CaSR is associated with lethal prostate cancer and the suggestive interactions with

Table 3. Global CaSR association and individual SNP associations (OR and 95% CI) for lethal prostate cancer by plasma 25(OH)D levels

<table>
<thead>
<tr>
<th>SNP name (minor, major allele)</th>
<th>Low plasma 25(OH)D (n = 73 lethal cases)</th>
<th>High plasma 25(OH)D (n = 40 lethal cases)</th>
<th>P interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>rs9820206(A,G)</td>
<td>0.68 (0.43–1.07)</td>
<td>0.09</td>
<td>1.20 (0.72–2.01)</td>
</tr>
<tr>
<td>rs6438705(A,G)</td>
<td>0.62 (0.37–1.06)</td>
<td>0.08</td>
<td>0.67 (0.33–1.36)</td>
</tr>
<tr>
<td>rs13083990(C,T)</td>
<td>0.60 (0.40–0.90)</td>
<td>0.01</td>
<td>0.74 (0.44–1.26)</td>
</tr>
<tr>
<td>rs4678173(A,C)</td>
<td>1.01 (0.71–1.45)</td>
<td>0.94</td>
<td>1.57 (1.00–2.47)</td>
</tr>
<tr>
<td>rs1979869(T,C)</td>
<td>0.59 (0.38–0.94)</td>
<td>0.03</td>
<td>0.98 (0.56–1.70)</td>
</tr>
<tr>
<td>rs2270916(C,T)</td>
<td>2.03 (1.32–3.11)</td>
<td>0.001</td>
<td>0.89 (0.45–1.75)</td>
</tr>
<tr>
<td>rs16832787(A,G)</td>
<td>1.31 (0.86–2.00)</td>
<td>0.22</td>
<td>1.08 (0.62–1.88)</td>
</tr>
<tr>
<td>rs2134223(G,A)</td>
<td>0.87 (0.53–1.43)</td>
<td>0.59</td>
<td>1.10 (0.57–2.12)</td>
</tr>
<tr>
<td>rs4638910(T,G)</td>
<td>1.19 (0.72–1.97)</td>
<td>0.50</td>
<td>0.64 (0.30–1.37)</td>
</tr>
<tr>
<td>rs1501900(T,A)</td>
<td>1.06 (0.69–1.61)</td>
<td>0.80</td>
<td>1.31 (0.77–2.23)</td>
</tr>
<tr>
<td>rs1393198(C,T)</td>
<td>0.89 (0.52–1.53)</td>
<td>0.68</td>
<td>1.05 (0.53–2.08)</td>
</tr>
<tr>
<td>rs7613238(G,A)</td>
<td>1.35 (0.94–1.93)</td>
<td>0.10</td>
<td>0.81 (0.50–1.29)</td>
</tr>
<tr>
<td>rs7637874(T,C)</td>
<td>1.44 (0.98–2.13)</td>
<td>0.06</td>
<td>0.87 (0.50–1.50)</td>
</tr>
<tr>
<td>rs7648041(T,C)</td>
<td>0.99 (0.67–1.46)</td>
<td>0.95</td>
<td>0.83 (0.48–1.42)</td>
</tr>
<tr>
<td>rs7630625(A,G)</td>
<td>1.06 (0.73–1.55)</td>
<td>0.76</td>
<td>0.93 (0.57–1.51)</td>
</tr>
<tr>
<td>rs1801725(T,G)</td>
<td>0.54 (0.31–0.95)</td>
<td>0.03</td>
<td>0.98 (0.52–1.87)</td>
</tr>
<tr>
<td>rs1042636(G,A)</td>
<td>0.80 (0.40–1.60)</td>
<td>0.54</td>
<td>0.75 (0.30–1.88)</td>
</tr>
<tr>
<td>rs1801726(G,C)</td>
<td>1.14 (0.55–2.37)</td>
<td>0.73</td>
<td>1.99 (0.72–5.53)</td>
</tr>
<tr>
<td>Kernel machine result</td>
<td>0.009</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

*aLow plasma 25(OH)D: 614 controls; high plasma 25(OH)D: 620 controls.*
plasma 25(OH)D and dietary calcium intake lend further support that calcium and vitamin D play a role in the development of lethal prostate cancer.

One nominally significant SNP (rs13083990) for lethal prostate cancer located in the 3’ area downstream from the gene was in partial LD ($r^2 = 0.36$) with rs1801725. The other SNPs which showed nominal significance with lethal prostate cancer were all intronic with unknown function and further studies are needed to follow-up on the potential causal variants should these results be replicated.

The only other case–control study to assess genetic variation in CaSR and prostate cancer risk was conducted in African American men and only genotyped the 3 nonsynonymous SNPs (rs1801725, rs1042636, and rs1801726). That study found that those homozygous for the variant allele of rs1801726 had a reduced risk of advanced prostate cancer (OR, 0.16; 95% CI, 0.03–0.74) compared with those homozygous for the major allele; they did not find an association with rs1801725. Our study did not find evidence for an association with rs1801726; however, the MAFs for these SNPs are significantly different in the Caucasian and African American populations. For example the MAF for rs1801726 was 0.04 for Caucasians compared with 0.18 for African Americans; the MAF for rs1801725 is 0.16 for Caucasians in our study and only 0.03 for African Americans.

A case-only GWAS of prostate cancer comparing lethal to indolent cases did not report any significant findings for SNPs in the CaSR gene (34). However, that study did not assess the joint effect of common variation across the entire CaSR gene and was not able to assess effect modification by calcium and plasma 25(OH)D. Finally, a GWAS study of advanced prostate cancer also did not identify any susceptibility loci in the CaSR gene (35). Importantly, the definition of advanced cancers in that study combined both Gleason score (8–10) and stage (T3 or higher), whereas our study specifically focused on the more definitive outcome of lethal cancer. Although Gleason score is an important predictor of prostate cancer progression, most cancers diagnosed at a high Gleason score will not progress to a lethal outcome and the overall positive predictive value of Gleason score for mortality is relatively low (36). There is also evidence that including cancers that are stage T3a at diagnosis is not an ideal surrogate for lethal outcomes (37). Our study did not find an association of variation in CaSR with prostate cancers defined by a Gleason of 7 (4 + 3) or higher. Furthermore, when we analyzed prostate cancer cases based on a stage at diagnosis of T3a and higher we also did not find an association (data not shown). Thus, it appears that the findings in our study were primarily driven by lethal cancers. It may be that CaSR acts independent of grade to drive progression to lethal cancers through

### Table 4. Global CaSR association and individual SNP associations (OR and 95% CI) for lethal prostate cancer by total calcium intake

<table>
<thead>
<tr>
<th>SNP name (minor, major allele)</th>
<th>Low calcium intake ($n = 74$ lethal cases)</th>
<th>High calcium intake ($n = 39$ lethal cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>rs9820206(A,G)</td>
<td>0.77 (0.50–1.17)</td>
<td>0.22</td>
</tr>
<tr>
<td>rs6438705(A,G)</td>
<td>0.66 (0.39–1.01)</td>
<td>0.11</td>
</tr>
<tr>
<td>rs13083990(C,T)</td>
<td>0.68 (0.46–1.01)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs4678173(A,C)</td>
<td>1.27 (0.90–1.78)</td>
<td>0.17</td>
</tr>
<tr>
<td>rs1979869(T,C)</td>
<td>0.65 (0.42–1.01)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs2270916(C,T)</td>
<td>1.54 (0.99–2.41)</td>
<td>0.06</td>
</tr>
<tr>
<td>rs16832787(A,G)</td>
<td>1.29 (0.86–1.92)</td>
<td>0.22</td>
</tr>
<tr>
<td>rs2134223(G,A)</td>
<td>0.96 (0.59–1.56)</td>
<td>0.85</td>
</tr>
<tr>
<td>rs4638910(G,T)</td>
<td>0.98 (0.59–1.62)</td>
<td>0.94</td>
</tr>
<tr>
<td>rs1501900(T,A)</td>
<td>1.04 (0.69–1.59)</td>
<td>0.85</td>
</tr>
<tr>
<td>rs1393198(C,T)</td>
<td>1.14 (0.69–1.88)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs7613238(G,A)</td>
<td>1.22 (0.86–1.73)</td>
<td>0.25</td>
</tr>
<tr>
<td>rs7637874(T,C)</td>
<td>1.62 (1.11–2.35)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs7648041(T,C)</td>
<td>1.12 (0.76–1.64)</td>
<td>0.56</td>
</tr>
<tr>
<td>rs7630625(A,G)</td>
<td>0.90 (0.62–1.30)</td>
<td>0.57</td>
</tr>
<tr>
<td>rs1801725(T,G)</td>
<td>0.63 (0.38–1.05)</td>
<td>0.08</td>
</tr>
<tr>
<td>rs1042636(G,A)</td>
<td>0.86 (0.45–1.65)</td>
<td>0.65</td>
</tr>
<tr>
<td>rs1801726(G,C)</td>
<td>1.47 (0.75–2.89)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Low calcium intake: 829 controls; high calcium intake: 415 controls.
influencing processes such as cell adhesion and metastases to bone. This is consistent with a study by Liao and colleagues (2006; ref. 13) which observed that elevated calcium acting through CaSR increased proliferation in prostate cancer cell lines with high metastatic potential, but not in the LNCaP cell line, which does not metastasize to bone.

**Strengths and limitations**

Our study was restricted to Caucasian men of European descent and these findings may not be generalizable to other ethnicities that are known to have different allele frequencies (38) and different prostate cancer risks. Although we adjusted for multiple testing at the gene-level using a global test, we cannot rule out that our results could be because of chance; however, the findings were biologically plausible and warrant further investigation in other cohorts for replication. Although a single measurement of plasma 25(OH)D has reasonable validity over a 5-year period (25, 39), multiple measurements over time would provide a better measure or long-term exposure and reduce the amount of nondifferential measurement error. Because blood samples were assayed at 4 different time points there was some batch-to-batch variation. However the rankings within each batch remained valid and we created batch-specific cut points in our analyses. Strengths of our study included the comprehensive linkage disequilibrium SNP selection method, the availability of dietary and supplement calcium intake data and plasma 25(OH)D levels, the ability to assess tumor characteristics by medical record review, and, importantly, the long follow-up for lethal outcomes.

**Conclusions**

In summary, our findings indicate that CaSR may play a role in prostate cancer progression perhaps via a mechanism involving bone remodeling and skeletal metastases. As bony metastases result in a high level of morbidity and mortality related to prostate cancer, gaining a better understanding the complex pathology of these bone lesions and the role of CaSR may lead to new insights and therapeutic strategies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

**Conception and design:** I.M. Shui, L.A. Mucci, E. Giovannucci

**Development of methodology:** I.M. Shui, E. Giovannucci

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** I.M. Shui, M.J. Stampfer, E. Giovannucci

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** I.M. Shui, L.A. Mucci, K.M. Wilson, P. Kraft, K.L. Penney

---

**Table 5. Global CaSR association and individual SNP results by prostate cancer subgroups**

<table>
<thead>
<tr>
<th>SNP name</th>
<th>Overall (n = 1193)</th>
<th>High grade (Gleason score 4 + 3 to 10; n = 225)</th>
<th>Advanced stage and lethal (n = 158)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>rs9820206(A,G)</td>
<td>1.00 (0.88-1.14)</td>
<td>0.95 (0.81-1.11)</td>
<td>1.05 (0.84-1.33)</td>
</tr>
<tr>
<td>rs6438705(A,G)</td>
<td>0.98 (0.84-1.13)</td>
<td>0.74 (0.60-1.01)</td>
<td>0.90 (0.69-1.18)</td>
</tr>
<tr>
<td>rs13083990(C,T)</td>
<td>0.97 (0.88-1.10)</td>
<td>0.67 (0.50-0.91)</td>
<td>0.94 (0.75-1.17)</td>
</tr>
<tr>
<td>rs4678173(A,C)</td>
<td>1.09 (0.97-1.22)</td>
<td>1.16 (0.99-1.34)</td>
<td>1.17 (0.95-1.44)</td>
</tr>
<tr>
<td>rs1979869(T,C)</td>
<td>0.97 (0.85-1.11)</td>
<td>0.68 (0.52-0.89)</td>
<td>1.02 (0.81-1.29)</td>
</tr>
<tr>
<td>rs2270916(T,C)</td>
<td>1.03 (0.87-1.21)</td>
<td>0.73 (0.59-0.90)</td>
<td>1.18 (0.89-1.56)</td>
</tr>
<tr>
<td>rs16832787(A,G)</td>
<td>0.99 (0.86-1.14)</td>
<td>0.88 (0.74-1.05)</td>
<td>0.96 (0.75-1.22)</td>
</tr>
<tr>
<td>rs2134223(G,A)</td>
<td>0.95 (0.81-1.12)</td>
<td>0.52 (0.38-0.71)</td>
<td>1.13 (0.86-1.49)</td>
</tr>
<tr>
<td>rs4638910(G,T)</td>
<td>0.98 (0.83-1.15)</td>
<td>0.80 (0.66-1.00)</td>
<td>0.85 (0.62-1.15)</td>
</tr>
<tr>
<td>rs1501900(T,A)</td>
<td>0.97 (0.84-1.11)</td>
<td>0.64 (0.50-0.84)</td>
<td>1.06 (0.83-1.36)</td>
</tr>
<tr>
<td>rs1393198(C,T)</td>
<td>1.00 (0.85-1.19)</td>
<td>0.96 (0.81-1.14)</td>
<td>0.89 (0.65-1.22)</td>
</tr>
<tr>
<td>rs7613238(G,A)</td>
<td>0.99 (0.88-1.11)</td>
<td>0.84 (0.70-1.01)</td>
<td>0.99 (0.81-1.21)</td>
</tr>
<tr>
<td>rs7637874(T,C)</td>
<td>1.12 (0.98-1.28)</td>
<td>0.90 (0.76-1.06)</td>
<td>1.07 (0.84-1.35)</td>
</tr>
<tr>
<td>rs7648041(T,C)</td>
<td>1.04 (0.91-1.18)</td>
<td>0.58 (0.42-0.79)</td>
<td>0.94 (0.75-1.18)</td>
</tr>
<tr>
<td>rs7630625(G,A)</td>
<td>0.90 (0.79-1.01)</td>
<td>0.07 (0.03-0.37)</td>
<td>0.98 (0.79-1.22)</td>
</tr>
<tr>
<td>rs1801725(T,G)</td>
<td>0.95 (0.81-1.12)</td>
<td>0.55 (0.41-0.74)</td>
<td>1.00 (0.76-1.32)</td>
</tr>
<tr>
<td>rs1042636(G,A)</td>
<td>0.91 (0.74-1.11)</td>
<td>0.35 (0.19-0.66)</td>
<td>1.10 (0.78-1.54)</td>
</tr>
<tr>
<td>rs1801726(G,C)</td>
<td>1.06 (0.80-1.40)</td>
<td>0.69 (0.50-0.95)</td>
<td>1.12 (0.69-1.81)</td>
</tr>
</tbody>
</table>

*All analyses use 1,244 controls.*
Writing, review, and/or revision of the manuscript: I.M. Shui, L.A. Mucci, K.M. Wilson, K.L. Penney, M.J. Stampfer, E. Giovannucci

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I.M. Shui, P. Kraft

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References


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Common Genetic Variation of the Calcium-Sensing Receptor and Lethal Prostate Cancer Risk

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