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## **Calcium channel blocker use is associated with a reduced risk of *TMPRSS2:ERG* gene fusion-positive prostate cancer**

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## **ABSTRACT**

### **Introduction**

Calcium channel blockers (CCBs) may affect prostate cancer (PCa) growth by modulating calcium-regulated tumorigenic processes. Recent epidemiological data showed etiological differences by molecular subtypes of PCa, in particular for PCa with the *TMPRSS2:ERG* (*T2E*) gene fusion. We studied the association between CCB use and risk of PCa overall and stratified by *T2E* status.

### **Methods**

Participants were residents of King County, Washington, recruited for population-based case-control studies (1993–1996 or 2002–2005). Tumor *T2E* status was determined by fluorescence in situ hybridization using tumor tissue samples from radical prostatectomy specimens. Detailed information on use of CCBs and other variables was obtained through in-person interviews. Binomial and polytomous logistic regression were used to generate odds ratios (ORs) and 95% confidence intervals (CIs).

### **Results**

The study includes 1,747 PCa patients and 1,635 age-matched controls. A subset of 563 radical prostatectomy patients had *T2E* status determined, of which 295 were *T2E* positive (52%). Use of CCBs (ever vs. never) was not associated with overall PCa risk. However, among European-American men, users had a reduced risk of higher-grade PCa (Gleason scores  $\geq 7$ : adjusted OR = 0.64; 95% CI: 0.44, 0.95). Further, use of CCBs was associated with a reduced risk of *T2E* positive PCa (adjusted OR = 0.38; 95% CI: 0.19, 0.78), but not *T2E* negative PCa.

### **Conclusion**

Use of CCBs was associated with reduced relative risks for higher Gleason score and *T2E* positive PCa. Future studies of PCa etiology should consider etiologic heterogeneity as PCa subtypes may have different causal pathways.

## INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed solid tumor in men (1). There is substantial heterogeneity among primary prostate cancers, evident in the spectrum of its molecular alterations and patients' variable prognosis (2). One of the most common somatic alterations in PCa is the gene fusion *TMPRSS2:ERG* (*T2E*), which is an early event in PCa that occurs in about half of all patients of European ancestry (3,4). There is substantial evidence that *T2E* positive PCa represents a distinct disease subtype with different underlying oncogenic pathways (2,5-8). Several recent epidemiological studies also showed etiological differences by *T2E* status identifying specific risk factors that were uniquely associated with *T2E* positive but not *T2E* negative disease (9-11).

Calcium channel blockers (CCBs) are used for the treatment of hypertension (12). Frequently prescribed CCBs include dihydropyridines (e.g., nifedipine), phenylalkylamines (e.g., verapamil), and benzothiazepines (e.g., diltiazem) (13,14). These drugs target calcium channels, which regulate calcium homeostasis (15,16), and may thereby alter cellular processes relevant to cancer including proliferation and apoptosis (16-18). Increased calcium channel activity has been associated with increased cellular proliferation and cancer growth (19,20). Many studies have identified alterations in the expression of calcium channel genes in cancer, including PCa (16,19,21). Different types of calcium channels have been implicated in prostate carcinogenesis including members of the ORAI, TRP, L-type and T-type family of channels (19,22-25). Further, there is some evidence of calcium channel genes being differentially expressed in prostate tumors that harbor the *T2E* fusion (5,8,22,26-28).

A number of prior epidemiological studies have investigated the association of CCB use with PCa risk (29). Two of the studies reported an inverse association (30,31), six studies found no evidence of an association (32-37), and one study showed a positive association (38). These prior studies, however, did not comprehensively investigate the association of CCB use with features of more aggressive PCa; and no prior study has examined the association stratified by molecular subtypes of PCa such as *T2E* gene fusion-positive disease.

In the present population-based study we investigated associations between CCB use and PCa risk. We examined associations with risk of overall and more aggressive PCa, and in men with *T2E* positive and *T2E* negative PCa.

## **METHODS**

### **Study population**

Study participants were residents of King County, Washington, who participated in population-based case–control studies (39,40). Incident cases diagnosed with histologically confirmed adenocarcinoma of the prostate were identified via the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. Cases from the first study (ages: 40 to 64 years) were diagnosed with PCa from 1993 through 1996 and cases from the second study (ages: 35 to 74 years) were diagnosed between 2002 and 2005. In total, 1,754 patients were available for the study. Data on Gleason score, diagnostic prostate-specific antigen (PSA) level, and tumor stage were collected from the cancer registry. Population-based controls without a history of PCa (n = 1,645) were identified using random digit telephone dialing, recruited evenly throughout the ascertainment periods for cases, and frequency-matched to cases by five-year age groups. All participants signed informed consent for participation, and the studies were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

### **Use of calcium channel blockers**

Information on lifetime use of CCBs was obtained during a detailed in-person interview. Medication show cards were used to obtain information on the type of CCB used. Seven cases and ten controls had missing data on CCB use and were excluded from the analysis. In addition, data on duration of use, time of first use, and current vs. former use were available for the subset of men in the second study, but not the men in the first study. Information on several other factors including demographic and lifestyle factors, personal and family medical history, and other cancer-related factors was collected during the interview.

### **Determination of *TMPRSS2:ERG* gene fusion status**

Formalin-fixed, paraffin-embedded tumor tissue blocks from radical prostatectomy specimens were used to make hematoxylin and eosin stained slides. These slides were reviewed by a prostate pathologist, who marked areas containing  $\geq 75\%$  tumor tissue. From these areas, two 1-mm tumor cores were taken and embedded in recipient paraffin blocks for the creation of tumor tissue microarrays. Fluorescence in situ hybridization (FISH) ‘break-apart’ assays were used to determine *T2E* status. A two-color FISH technique was used as described previously (9). In total, 563 patients had *T2E* status available for analysis.

## Statistical data analysis

Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between ever vs. never use of CCBs and PCa risk. Polytomous logistic regression was used for the analysis in which patients were stratified by tumor *T2E* status and Gleason score (<7 vs. ≥7). The statistical models were adjusted for age (five-year age groups) and race (African-American, European-American). Other variables were also considered as potential confounders: education, body mass index, lifetime alcohol consumption, aspirin and other NSAIDs use, recent exercise frequency, history of diabetes mellitus, and history of hypertension. We investigated the effect of adding these variables to the model, one by one, on the OR for CCB use. Only body mass index (continuous) and history of hypertension (no, yes) changed the OR by at least 5%; and these variables were therefore additionally included in the final model. Associations were also evaluated in the subgroup of European-American men. African-American patients were not considered separately because the numbers were too small. All *P*-values were two-sided and a *P*-value of less than 0.05 was considered to be statistically significant. All statistical analyses were conducted using the R programming language.

## RESULTS

The study population included 1,747 PCa patients and 1,635 controls (Table 1). As expected, patients were more likely to have a first-degree family history of PCa compared to controls. Tumor tissue was collected for the patients who had a radical prostatectomy as their primary treatment for PCa, which was used to determine *T2E* status. Of the 563 patients with *T2E* data, 295 (52%) had tumors that were *T2E* gene fusion-positive. Positive fusion status was associated with younger ages at diagnosis and European-American ancestry. Patients with *T2E* positive tumors had lower Gleason scores. Almost all CCB users in the study used drugs from the following drug classes: dihydropyridines (52.9%), phenylalkylamines (24.8%), and benzothiazepines (21.8%).

The frequency of any CCB use among controls was 8.6% (Table 2). Use of CCBs was not associated with the relative risk of overall, lower-grade (Gleason <7), or higher-grade (Gleason  $\geq$ 7) PCa with adjusted ORs of 0.87 (95% CI: 0.67, 1.13), 0.99 (95% CI: 0.73, 1.33), and 0.74 (95% CI: 0.53, 1.04), respectively (Table 2). However, in the subgroup of European-American men, use of CCBs was inversely associated with risk of higher-grade PCa (adjusted OR = 0.64; 95% CI: 0.44, 0.95). The association was more pronounced for Gleason  $\geq$ 7(4+3) PCa revealing an adjusted OR of 0.53 (95% CI: 0.29, 0.98). There was no evidence suggesting that the associations differ substantially by type of CCB. For a subset of men, data were available on current vs. former use, duration of use, and time since first use of CCBs. No significant associations were found in these subgroups (Table 3). In the subgroup of European-American men, however, current use of CCBs was associated with a lower risk of higher-grade PCa (adjusted OR = 0.44, 95% CI: 0.23, 0.83).

Table 4 shows the association between CCB use and PCa risk stratified by *T2E* status. Use of CCBs was associated with a reduced risk of *T2E* positive PCa with an adjusted OR of 0.38 (95% CI: 0.19, 0.78). Of the *T2E* positive PCa patients that were CCB users, 78% (7 out of 9) had lower-grade disease (Gleason scores  $\leq$ 6). No association was observed for *T2E* negative PCa (adjusted OR = 1.05; 95% CI: 0.65, 1.69). The association of CCB use with fusion-positive PCa was slightly more pronounced when the analysis was restricted to European-American men (adjusted OR = 0.33; 95% CI: 0.15, 0.73). Among the subset of European-American men, all *T2E* positive PCa patients who used CCBs (n = 7) had lower-grade disease (Gleason scores  $\leq$ 6).

## DISCUSSION

This PCa case–control study showed that ever use of CCBs was associated with a decreased risk in patients whose tumors were positive for the *T2E* gene fusion, which is an important molecular subtype of PCa. No associations were observed between CCB use and risk of *T2E* negative PCa. In the larger study population there was no association between CCB use and overall PCa risk, however, among European-American men CCB use was inversely associated with risk of higher-grade PCa.

Although prior studies have not investigated the association between CCB use and PCa risk in patients stratified by tumor *T2E* status; at least nine observational studies of CCBs and overall PCa risk have been conducted (29,32,37). Of these, two small prospective studies found an inverse association. The first study by Fitzpatrick et al. included 2,442 men (151 PCa patients), and found that CCB users had a relative risk of PCa of 0.6 (95% CI: 0.4, 0.9) (30). The second study by Debes et al. included 1,362 men (135 PCa patients), and showed that CCB users had a relative risk of PCa of 0.55 (95% CI: 0.31, 0.97) (31). The majority of studies, however, found no association between CCB use and PCa risk. These investigations include three cohort studies (2 prospective and 1 retrospective study) (32,34,37), including a prospective cohort study with 3,031 incident PCa patients (34), and four case–control studies (33,35,36,38). Only one previous observational study specifically investigated CCB use in relation to more aggressive PCa features, and the study found no association (34). Based on our results showing an inverse association between CCB use and higher-grade PCa, further studies are needed to examine whether CCBs might be associated with a reduced risk of more clinically aggressive forms of PCa. Our study also showed novel evidence that CCBs may reduce the risk of *T2E* positive PCa. Given that this is the first study to examine the association; further molecular epidemiological studies focused on CCB use in patients stratified by *T2E* tumor subtypes are needed.

Calcium channels regulate calcium homeostasis and are involved in several important cellular mechanisms (16,19). Altered expression of calcium channel genes is common in cancer, and increased calcium channel activity can increase cellular proliferation and may promote PCa growth (21). Overexpression of specific calcium channels genes may also protect PCa cells from apoptosis (23,41). Although the precise biological mechanisms through which CCBs may reduce risk of *T2E* positive PCa are unclear, one possible mechanism might involve the calcium channel gene *CACNA1D*, which is known to be highly overexpressed in *T2E* positive PCa (5,8,22,26-28). Previous evidence from our group showed that this increase in *CACNA1D* expression may result from aberrant tumor DNA methylation of the gene (8). *CACNA1D* encodes the calcium-channel, voltage-dependent, L-type, alpha 1D subunit, Cav1.3 (42).



This L-type calcium channel is a target of the most commonly prescribed CCBs including dihydropyridines, phenylalkylamines, and benzothiazepines (13,14). Further, it has been shown that suppression of *CACNA1D* inhibits androgen-stimulated calcium influx, androgen receptor transactivation, and PCa cell growth (22). Additional mechanistic studies are needed to fully understand the potential link between use of CCBs and development of *T2E* positive tumors, and the potential involvement of the *CACNA1D* gene.

Strengths of this study include its population-based design, availability of data on many potential confounding variables, and data on tumor *T2E* gene fusion status determined using fluorescence in situ hybridization, which is considered the 'gold standard' for assessing the gene fusion. Because of the retrospective study design, the possibility of recall bias in relation to medication use cannot be ruled out. However, we would not expect recall to vary by tumor molecular subtype, which is therefore unlikely to have impacted our findings related to *T2E* status. Another potential limitation is that tumor *T2E* status was only available for the subset of patients who chose radical prostatectomy for primary treatment. Finally, although we adjusted for many potential confounders, residual confounding might be a problem. For example, use of CCBs is clearly a marker for the presence of high blood pressure, which is linked to obesity and cardio-metabolic abnormalities that may increase cancer incidence. That would, however, not explain the reduced relative risk observed in our study.

In conclusion, the present study showed that CCB use was associated with a lower risk of an important molecular subtype of PCa, *T2E* gene fusion-positive disease. If confirmed, our results suggest that CCBs, which are commonly used for the treatment of hypertension, may also reduce the risk of developing *T2E* positive PCa, a molecular subtype of PCa that represents approximately half of all prostate tumors diagnosed in men of European ancestry. This molecular epidemiological study of PCa highlights the importance of examining molecular subtypes when studying cancer risk or protective factors.

## **NOTES**

### **Conflict of interest**

The authors declare that they have no conflict of interest.

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**Table 1**

Characteristics of study participants, King County, WA, 1993–1996 and 2002–2005.

Variables	Controls (n = 1,635)			Overall PCa cases (n = 1,747)			T2E negative PCa cases (n = 268)			T2E positive PCa cases (n = 295)		
	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%
Age (years)	59.2 (7.2)	–	–	59.8 (7.0)	–	–	59.3 (6.9)	–	–	56.9 (6.6)	–	–
Race												
African-American	–	114	7.0%	–	205	11.7%	–	30	11.2%	–	15	5.1%
European-American	–	1,521	93.0%	–	1,542	88.3%	–	238	88.8%	–	280	94.9%
First-degree family history of PCa												
No	–	1,457	89.1%	–	1,381	79.0%	–	204	76.1%	–	232	78.6%
Yes	–	178	10.9%	–	366	21.0%	–	64	23.9%	–	63	21.4%
Body mass index (kg/m <sup>2</sup> )	27.2 (4.2)	–	–	27.1 (4.1)	–	–	26.9 (3.7)	–	–	26.7 (3.7)	–	–
Smoking status												
Never	–	690	42.2%	–	705	40.4%	–	112	41.8%	–	128	43.4%
Former	–	713	43.6%	–	808	46.3%	–	133	49.6%	–	135	45.8%
Current	–	232	14.2%	–	234	13.4%	–	23	8.6%	–	32	10.9%
History of hypertension												
No	–	1,125	68.9%	–	1,122	64.2%	–	175	65.3%	–	206	69.8%
Yes	–	508	31.1%	–	625	35.8%	–	93	34.7%	–	89	30.2%
Gleason score												
≤6	–	–	–	–	983	56.5%	–	117	43.7%	–	161	54.6%
7(3+4)	–	–	–	–	478	27.5%	–	96	35.8%	–	100	33.9%
7(4+3)	–	–	–	–	113	6.5%	–	32	11.9%	–	15	5.1%
8–10	–	–	–	–	165	9.5%	–	23	8.6%	–	19	6.4%
Disease stage												
Local	–	–	–	–	1,366	78.2%	–	186	69.4%	–	200	67.8%
Regional	–	–	–	–	334	19.1%	–	81	30.2%	–	95	32.2%
Distant	–	–	–	–	47	2.7%	–	1	0.4%	–	0	0.0%

PSA (ng/mL) at diagnosis												
<4	–	–	–	–	227	14.1%	–	33	13.2%	–	54	19.4%
4–<10	–	–	–	–	970	60.2%	–	156	62.2%	–	176	63.3%
10–<20	–	–	–	–	250	15.5%	–	45	17.9%	–	31	11.2%
≥20	–	–	–	–	163	10.1%	–	17	6.8%	–	17	6.1%
Primary treatment												
Radical prostatectomy	–	–	–	–	984	56.1%	–	268	100.0%	–	295	100.0%
Radiation with or without ADT	–	–	–	–	508	29.0%	–	–	–	–	–	–
ADT only	–	–	–	–	91	5.2%	–	–	–	–	–	–
Active surveillance	–	–	–	–	164	9.4%	–	–	–	–	–	–
Other	–	–	–	–	7	0.4%	–	–	–	–	–	–

Note: ADT: androgen-deprivation therapy; PCa, prostate cancer; PSA, prostate-specific antigen; SD, standard deviation; *T2E*, *TMPRSS2:ERG*.

**Table 2**

Associations between calcium channel blocker use and risk of overall, lower-grade, and higher-grade prostate cancer, King County, WA, 1993–1996 and 2002–2005.

	Calcium channel blocker use		
	No	Yes	
	OR (ref)	OR	(95% CI)
<i>All men</i>			
No. controls	1,495	140	
Overall PCa			
No. cases	1,598	149	
Age-adjusted	1.00	0.97	(0.76, 1.24)
Multivariable-adjusted <sup>a</sup>	1.00	0.87	(0.67, 1.13)
Gleason score <7			
No. cases	896	87	
Age-adjusted	1.00	1.05	(0.79, 1.39)
Multivariable-adjusted <sup>a</sup>	1.00	0.99	(0.73, 1.33)
Gleason score ≥7			
No. cases	695	61	
Age-adjusted	1.00	0.78	(0.57, 1.08)
Multivariable-adjusted <sup>a</sup>	1.00	0.74	(0.53, 1.04)
<i>European-American men</i>			
No. controls	1,397	124	
Overall PCa			
No. cases	1,426	116	
Age-adjusted	1.00	0.91	(0.70, 1.19)
Multivariable-adjusted <sup>a</sup>	1.00	0.84	(0.63, 1.12)
Gleason score <7			
No. cases	810	75	
Age-adjusted	1.00	1.08	(0.80, 1.46)
Multivariable-adjusted <sup>a</sup>	1.00	1.00	(0.72, 1.38)
Gleason score ≥7			
No. cases	611	40	
Age-adjusted	1.00	0.69	(0.48, 1.00)
Multivariable-adjusted <sup>a</sup>	1.00	0.64	(0.44, 0.95)

Note: CI, confidence interval; OR, odds ratio; PCa, prostate cancer.

<sup>a</sup> Models were adjusted for age (five-year age groups), race (African-American, European-American), body mass index (continuous), and history of hypertension (no, yes).



**Table 3**

Odds ratio and 95% confidence interval for prostate cancer risk by categories of calcium channel blocker use, King County, WA, 2002–2005<sup>a</sup>.

	All men				European-American men			
	No. controls	No. cases	OR	(95% CI)	No. controls	No. cases	OR	(95% CI)
<i>Overall PCa</i>								
Use								
Never (ref.)	844	901	1.00		757	768	1.00	
Former	31	35	1.00	(0.60, 1.66)	29	31	1.00	(0.59, 1.70)
Current	67	65	0.82	(0.56, 1.19)	58	44	0.71	(0.47, 1.09)
Duration of use, years								
<5	37	41	0.97	(0.61, 1.56)	31	34	1.02	(0.61, 1.70)
≥5	54	56	0.87	(0.58, 1.31)	49	38	0.73	(0.47, 1.15)
Time since first use, years								
<5	28	27	0.84	(0.48, 1.45)	23	23	0.93	(0.51, 1.69)
≥5	60	67	0.94	(0.64, 1.38)	56	46	0.76	(0.51, 1.17)
<i>Gleason score &lt;7</i>								
Use								
Never (ref.)	844	469			757	407	1.00	
Former	31	18	1.03	(0.56, 1.89)	29	15	0.94	(0.49, 1.81)
Current	67	38	0.99	(0.64, 1.53)	58	30	0.93	(0.58, 1.51)
Duration of use, years								
<5	37	27	1.27	(0.75, 2.15)	31	23	1.32	(0.74, 2.34)
≥5	54	27	0.88	(0.54, 1.45)	49	20	0.75	(0.43, 1.31)
Time since first use, years								
<5	28	19	1.18	(0.64, 2.16)	23	17	1.32	(0.69, 2.55)
≥5	60	35	1.03	(0.66, 1.63)	56	26	0.84	(0.51, 1.40)
<i>Gleason score ≥7</i>								
Use								
Never (ref.)	844	428			757	359	1.00	
Former	31	17	0.99	(0.53, 1.84)	29	16	1.06	(0.56, 2.01)
Current	67	26	0.63	(0.39, 1.03)	58	13	0.44	(0.23, 0.83)
Duration of use, years								
<5	37	14	0.66	(0.34, 1.25)	31	11	0.70	(0.34, 1.43)
≥5	54	28	0.86	(0.53, 1.41)	49	17	0.68	(0.38, 1.21)
Time since first use, years								
<5	28	8	0.49	(0.22, 1.10)	23	6	0.52	(0.21, 1.29)
≥5	60	31	0.85	(0.53, 1.37)	56	19	0.65	(0.37, 1.13)

Note: CI, confidence interval; OR, odds ratio; PCa, prostate cancer.

<sup>a</sup> Models were adjusted for age (five-year age groups), race (African-American, European-American), body mass index (continuous), and history of hypertension (no, yes).

**Table 4**

Associations between calcium channel blocker use and risk of prostate cancer stratified by *TMPRSS2:ERG* gene fusion status, King County, WA, 1993–1996 and 2002–2005.

	Calcium channel blocker use		
	No	Yes	
	OR (ref)	OR	(95% CI)
<i>All men</i>			
No. controls	1,495	140	
<i>T2E positive PCa</i>			
No. cases	286	9	
Age-adjusted	1.00	0.40	(0.20, 0.79)
Multivariable-adjusted <sup>a</sup>	1.00	0.38	(0.19, 0.78)
<i>T2E negative PCa</i>			
No. cases	243	25	
Age-adjusted	1.00	1.11	(0.71, 1.75)
Multivariable-adjusted <sup>a</sup>	1.00	1.05	(0.65, 1.69)
<i>European-American men</i>			
No. controls	1,397	124	
<i>T2E positive PCa</i>			
No. cases	273	7	
Age-adjusted	1.00	0.36	(0.16, 0.78)
Multivariable-adjusted <sup>a</sup>	1.00	0.33	(0.15, 0.73)
<i>T2E negative PCa</i>			
No. cases	219	19	
Age-adjusted	1.00	1.00	(0.60, 1.66)
Multivariable-adjusted <sup>a</sup>	1.00	0.99	(0.58, 1.71)

Note: CI, confidence interval; OR, odds ratio; PCa, prostate cancer; *T2E*, *TMPRSS2:ERG*.

<sup>a</sup> Models were adjusted for age (five-year age groups), race (African-American, European-American), body mass index (continuous), and history of hypertension (no, yes).