HHS Public Access

Author manuscript

Prostate Cancer Prostatic Dis. Author manuscript; available in PMC 2016 July 12.

Published in final edited form as:

Prostate Cancer Prostatic Dis. 2016 March ; 19(1): 53–56. doi:10.1038/pcan.2015.49.

ASPIRIN AND NSAID USE IN ASSOCIATION WITH MOLECULAR SUBTYPES OF PROSTATE CANCER DEFINED BY *TMPRSS2:ERG* FUSION STATUS

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Abstract

Background—The *TMPRSS2:ERG* (*T2E*) gene fusion is the most common rearrangement in prostate cancer (PCa). It is unknown if these molecular subtypes have a different etiology. We evaluated aspirin and non-aspirin NSAIDs in association with *T2E* fusion status.

Methods—Subjects were from a population-based case-control study of PCa. *T2E* fusion status for prostatectomy cases (n=346) was determined by FISH. Medication use was determined from questionnaires. Logistic regression, controlling for age, race, PCa family history, and PSA screening, was used to evaluate the association of *T2E* fusion status according to medication use.

Results—*T2E* fusion was present in 171 (49%) cases, with younger cases more likely to be fusion positive ($p<0.01$). Current aspirin use was associated with a 37% risk reduction of *T2E* positive tumors (adjusted OR 0.63, 95% CI 0.43–0.93). Aspirin use was not associated with *T2E* negative PCa (adjusted OR 0.99, 0.69–1.42). There were no associations between PCa fusion status and use of non-aspirin NSAIDs or acetaminophen.

Conclusion—Aspirin is associated with a significant reduction in the relative risk of *T2E* fusion positive, but not *T2E* negative, PCa. Since inflammation and androgen pathways are implicated in prostate carcinogenesis, additional studies of anti-inflammatory medications in relation to these PCa subtypes are warranted.

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CONFLICTS OF INTERESTS:

There are no potential conflicts of interest.

Keywords

Prostate cancer; Aspirin; *TMPRSS2:ERG fusion*; Relative Risk

Introduction

Prostate cancer is a common malignancy, and there is interest in identifying potential chemoprevention agents. The disease is also heterogeneous in terms of its clinical features and biological behavior, and numerous strategies have been examined for their ability to stratify more homogeneous subsets of patients. The *TMPRSS2:ERG* (*T2E*) gene fusion is the most common somatic gene rearrangement in prostate cancer (PCa), found in approximately 50% of PCa cases.[1,2,3,4] The gene fusion is between *TMPRSS2*, a gene encoding a serine protease whose expression is regulated by androgens, and *ERG*, a known oncogene involved in cell proliferation. The fusion of these two genes results in enhanced androgen stimulation of the *ERG* oncogene.[1]

Aspirin (ASA) has been found to be consistently associated with a lower risk for development of PCa. [5,6,7,8,9,10] In a prior analysis we reported a modest reduction in the relative risk for PCa associated with aspirin use, but did not consider the cases' *T2E* fusion status.[5] To date, there have been few studies evaluating whether environmental or genetic factors are associated with *T2E* gene fusion status.[11] Information on the interplay of *T2E* fusion, prostate cancer and aspirin use may also shed light on the etiology of PCa. The exact mechanism of the inverse association between aspirin use and the development of PCa has not been completely elucidated. However, aspirin functions as an anti-inflammatory medication,[12] and there is an established relationship between inflammation and PCa.[13,14] In this analysis, we stratified PCa cases on the basis of *T2E* fusion status to assess whether the association with aspirin or other NSAIDs use differed in subgroups defined by this somatic change. Non-aspirin NSAIDs and acetaminophen were examined to determine if the anti-inflammatory effects were for the entire class of anti-inflammatory medications, or if the effects were aspirin specific.

Methods

Study Population

The study population is derived from men who participated in a prior population-based case-control study of PCa.[15] Cases were residents of King County, Washington with histologically confirmed PCa (identified from the Seattle-Puget Sound SEER cancer registry). Incident cases were diagnosed between January 1, 2002, and December 31, 2005. Cases included in this analysis were those who underwent radical prostatectomy and consented to collection of tissue, which was used to make tumor microarrays. Male residents of King County, Washington with no history of PCa were recruited as controls and identified using random digit telephone dialing. Controls were frequency matched to cases by five-year age groups and enrolled evenly throughout the study period.

Data Collection

In-person interviews were conducted by trained staff for collecting information on demographic and lifestyle factors, medical and family history, and PCa screening history (PSA and digital rectal exam (DRE)). Body mass index (BMI) was determined from self-reported height and weight (one year prior to reference date: date of diagnosis for cases and a randomly assigned date for controls that approximated the distribution of cases' diagnosis dates).

The study questionnaire also queried details of specific classes of medication usage, including dates of use and duration of use for each episode of use. Participants were provided a comprehensive list of medications containing aspirin, non-aspirin NSAIDs and acetaminophen (both prescription and over-the-counter) and asked whether they had ever used any of the medications at least once a week for three months or longer. Participants where then asked for start and end dates of each aspirin, non-aspirin NSAID or acetaminophen containing medication they reported using on a regular basis. Alternatively, participants could provide age of starting or stopping an aspirin, non-aspirin NSAID or acetaminophen containing medication. Current use was defined as use at the reference date. Former use was defined as use for at least once a week for three months or longer, but not at the reference date. Duration of aspirin, non-aspirin NSAID or acetaminophen use was then determined for each individual based on these data. First, only those who were current users of aspirin, non-aspirin NSAIDs or acetaminophen were considered. Men were then grouped as never users, former users, current users of < 5 years, and current users of ≥ 5 years duration. Five years was chosen as the cut point as that was the median reported duration of use for controls.

Construction of TMAs

FFPE tumor tissue blocks from radical prostatectomy samples were used to make H&E slides, which were reviewed by a prostate pathologist who marked areas containing >=75% tumor tissue. Two 1-mm tumor tissue cores were taken from these areas and embedded in recipient blocks for construction of TMAs.

Fluorescence in-situ hybridization

Identification of the *T2E* genetic rearrangement was determined using an *ERG* gene 'break-apart' assay as previously described.[16] Probe labeling was performed by random priming and a two-color FISH technique was used. Goat anti-FITC Alexa488 antibodies were used to amplify the green FITC signals. Pictures were acquired using Axioplan 2 Imaging System with Metafer Software. DAPI-Prescan (10× magnification) of the whole TMA slide was used to identify the core positions. Core identification numbers were assigned using a TMA tool implemented in Metafer. Each spot was scanned at 40× magnification, in a grid of $6 \times 9 = 54$ fields. Each field was photographed in at least three different focus planes with filters for FITC and Cy3. Referring layer and filter captures were then merged into one final three-colored image per field. After undergoing hybridization, each tissue core was evaluated by two separate individuals to determine if the specimen was *T2E* positive or negative. If there was disagreement, the specimen underwent further review until consensus was reached.

Cores with less than 25% evaluable material were excluded. Cores were considered positive if multiple cells contained the *T2E* rearrangement.

Statistical Analysis

Demographic and pathologic characteristics were compared between cases with and without *T2E* fusion status with Chi-squared tests. Polytomous logistic regression was performed to determine the odds ratio as an estimate of the relative risk for the two case groups defined by *T2E* fusion status to controls. Age adjusted and multivariate models were performed with aspirin, non-aspirin NSAID or acetaminophen use as the exposure of interest. Variables adjusted for in the multivariate model included age at reference date, race, first-degree family history of PCa, PCa screening within 5 years prior to diagnosis (cases) or referent date (controls). Odds ratios (OR) and 95% confidence intervals (95% CI) are reported. All analyses were performed with Stata SE/12 (College Station, TX).

Results

Tumor tissue was available for 346 cases and the *T2E* fusion was present in 171 (49%). Differences in selected demographic, environmental/lifestyle, medical and tumor characteristics are shown in Table 1. The proportion of PCa cases with the *T2E* fusion declined with age ($p < 0.01$). In cases under the age of 50, 66% were fusion positive, compared to 24% of men ages 70 – 74. The presence of the fusion was more commonly seen in men with lower grade tumors (52% and 53% of Gleason 2–6 and Gleason 3+4 tumors) than in higher grade tumors (26% and 44% of Gleason 4+3 and Gleason 8–10 tumors), respectively, $p = 0.03$. A family history of PCa, smoking status, BMI (grouped by the World Health Organization categories) and PSA screening history were not associated with fusion status in this data set. The demographics and other features of the controls (n = 942) are also shown in Table 1.

Table 2 shows the odds ratios for PCa stratified by *T2E* fusion status compared to controls according to aspirin, non-aspirin NSAIDs and acetaminophen usage. Current use of aspirin was reported by 30% of cases with fusion positive tumors, and the multivariate-adjusted OR for aspirin use was 0.63 (95% CI 0.43–0.93), corresponding to a 37% risk reduction compared to controls. Current use of aspirin was similar between controls (45%) and those with fusion negative tumors (42%). For fusion negative tumors, the multivariate-adjusted OR for aspirin use was 0.99 (95% CI 0.69–1.42). Current use of non-aspirin NSAIDs was similarly reported between controls (13%), those with fusion negative tumors (14%), and those with fusion positive tumors (9%). For fusion negative tumors, the multivariate-adjusted OR for non-aspirin NSAID use was 1.04, 95% CI 0.64–1.67. For fusion positive tumors, the multivariate-adjusted OR for non-aspirin NSAID use was 0.65, 95% CI 0.37–1.14. Current use of acetaminophen was similarly reported between controls (6%), those with fusion negative tumors (7%), and those with fusion positive tumors (5%). For fusion negative tumors, the multivariate-adjusted OR for acetaminophen use was 1.12, 95% CI 0.58–2.18. For fusion positive tumors, the multivariate-adjusted OR for acetaminophen use was 0.93, 95% CI 0.43–1.98.

Given the association between aspirin use and PCa risk, we further explored duration of aspirin use (Table 3). There was a duration effect response for aspirin use in *T2E* fusion positive tumors. When compared to those who never used aspirin, the risk reduction for *T2E* fusion positive tumors for current users with greater than 10 years of aspirin use (multivariate-adjusted OR 0.54, 95% CI 0.30–0.98) was greater than the risk reduction for *T2E* fusion positive tumors for current users with 5 to 9.9 years of aspirin use (multivariate-adjusted OR 0.54, 95% CI 0.30–0.98). There was a non-significant risk reduction for *T2E* fusion positive tumors for current users with less than 5 years of aspirin use. There was no association found between *T2E* fusion negative tumors for any duration of aspirin use.

Discussion

This study identified an association between aspirin use and a reduction in the risk of *T2E* positive PCa. Among men reporting current aspirin use, there was a 37% reduction in the risk of developing *T2E* positive tumors (OR 0.63, 95% CI 0.43 – 0.93), with a stronger risk reduction observed in relation to longer durations of use. No association with aspirin use was seen in *T2E* negative PCa.

The *T2E* gene fusion is the most common somatic gene rearrangement seen in PCa. It has been shown to be present in 46 – 70% of prostate tumors.[1,2,3,4] *TMPRSS2* is a gene that is up-regulated by androgen stimulation. *ERG* is a transcription factor that results in cell proliferation. The gene fusion causes androgen stimulation to drive cell proliferation. In this study, the *T2E* gene fusion was present in 49% of cases, consistent with other studies. [1,2,3,4] A change in the prevalence of *T2E* positive PCa and age was noted. Schaefer et al also found a decrease in the prevalence of *T2E* positive PCa with increasing age.[17] They observed the *T2E* fusion in 64% of men under age 56 with PCa. This percentage was reduced to 41% in men ages 67 and older. In our study we observed a similar trend, with 66% of men younger than 50 having the *T2E* fusion and 24% of men ages 70–74 having the *T2E* fusion ($p < 0.01$). This may imply that a different molecular pathway is responsible for the development of PCa in younger vs. older men.

Genomic rearrangement is the mechanism responsible for the development of the *T2E* fusion. There are several events that are necessary for genomic rearrangement to occur. First, the genes to be rearranged must be in close proximity; second, there must be double strand DNA breaks; and third, there must be improper repair of those breaks. The *TMPRSS2* and *ERG* genes are found approximately three Mb apart on chromosome 21. DNA double strand breaks are a regular occurrence within the nucleus, some of which are caused by the oxidative stress, DNA damage and genomic instability produced by reactive oxygen species (ROS).[18,19,20,21] Increased levels of ROS have been found in PCa cells.[22] ROS have also been implicated in the recruitment of leukocytes, which are necessary for initiation of an inflammatory response.[23] Inflammation has been identified as a factor responsible for the transformation of normal prostate epithelium to prostate cancer.[13,14]

There is also a relationship between androgens and inflammation. Androgens have been shown to increase inflammation during in vivo studies of non-prostate tissue.[24,25,26] Prostate specimens displaying inflammation have also been shown to have higher expression of the

androgen receptor.[27] Ripple et al demonstrated that androgens can result in oxidative stress in prostate cancer cells.[28] Interestingly, androgen receptor levels have been shown to be elevated in PCa found in younger individuals.[29] Both *T2E* fusion positive tumors and androgen receptor levels being increased in younger prostate cancer patients suggest that androgens play a role in the development of *T2E* positive prostate cancer.

Aspirin has long been established as an anti-inflammatory medication.[12] It has also been shown to decrease the amount of reactive oxygen species present within a cell.[30,31] This decrease in ROS would reduce the number of double stranded DNA breaks, and reduce the recruitment of mediators of the inflammatory pathway. In this way, aspirin use may limit the occurrence of the precursor events necessary for prostate tumorigenesis. This is a plausible mechanism as to the observed risk reduction of *T2E* positive PCa in relation to aspirin use.

Investigations of the association between aspirin use and the risk of developing PCa have shown conflicting results. Some studies have found evidence of a decreased risk of PCa for men taking aspirin,[5,6,7,8,9] whereas other studies have not found any association.[32,33] The results of our study may help explain some of these conflicting results. If aspirin reduces risk of *T2E* positive PCa, but not *T2E* negative PCa, the results of the previous studies may vary based on the *T2E* gene fusion status of the patients included. None of the previous studies examining the association between PCa and aspirin use have utilized molecular subtyping such as *T2E* gene fusion status to stratify patients.

To our knowledge this is the first study to report an association between aspirin use and a reduction in the risk of *T2E* positive PCa. Further studies are needed to determine if our results are reproducible, and to identify any other potential etiological factors associated with *T2E* positive versus negative PCa. The limitations of this study include self-reported aspirin use, which is subject to recall bias. However, because aspirin is available over-the-counter, self-report is necessary for capturing this exposure information. Cases included in the analysis consist of men who underwent surgery as primary treatment for PCa, and thus we do not have *T2E* status for patients who chose active surveillance or radiation treatment. There may be unmeasured variables that affect whether a patient is taking aspirin and which treatment modality is chosen. Further efforts to analyze *T2E* status on biopsy specimens would be informative.

In conclusion, our data indicate that aspirin use is associated with a decreased risk of developing *T2E* positive PCa, but is not associated with *T2E* negative PCa. Potentially, through a reduction in cellular stress and inflammation, aspirin use may protect against DNA strand breaks that are necessary for *T2E* fusion. Further studies are needed to confirm our results and determine the potential role of aspirin as a chemopreventive agent for *T2E* positive PCa.

Acknowledgments

RESEARCH SUPPORT:

NIH Grants: R01 CA 092579 (J Stanford); P50 CA097186 (J Stanford and D Lin); T32 CA009168-30 (J Wright); with additional support from the Fred Hutchinson Cancer Research Center, the Prostate Cancer Foundation, and the many men who generously participated in the study.

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Table 1

Distribution of Selected Factors in Controls and in Prostate Cancer Patients Stratified by TMPRSS2:ERG Fusion Status

	TMPRSS2:ERG Fusion Status			
	Controls	**Negative**	**Positive**	**P-value***
	N (%)	N (%)	N (%)	
Total	942(100)	175 (100)	171 (100)	
Age				
35 -- 49	93 (10)	20 (11)	38 (22)	< 0.01
50 -- 54	113 (12)	25 (14)	25 (15)	
55 -- 59	174 (18)	33 (19)	36 (21)	
60 -- 64	187 (20)	42 (24)	43 (25)	
65 -- 69	202 (21)	33 (19)	22 (13)	
≥ 70	170 (18)	22 (13)	7 (4)	
Race				
Caucasian	844 (90)	150 (86)	157 (92)	0.07
African American	98 (10)	25 (14)	14 (8)	
Family History of Prostate Cancer				
No	833 (88)	128 (73)	130 (76)	0.54
Yes	109 (12)	47 (27)	41 (24)	
Body Mass Index				
< 25.0	259 (27)	53 (30)	55 (32)	0.87
25.0 -- 29.0	444 (47)	92 (53)	85 (50)	
≥ 30.0	239 (25)	30 (17)	31 (18)	
Smoking Status				
Non-smoker	429 (46)	78 (45)	81 (47)	0.8
Former smoker	394 (42)	86 (49)	78 (46)	
Current smoker	118 (13)	11 (6)	12 (7)	
PSA tests in the 5 years preceding reference date				
None	231 (30)	31 (19)	44 (27)	0.14
1 -- 2	168 (22)	30 (18)	26 (16)	
3 -- 4	129 (17)	32 (20)	40 (24)	
≥ 5	251 (32)	70 (43)	54 (33)	
Diagnostic PSA**				
< 4.0		21 (13)	38 (23)	0.03
4.0 -- 9.9		110 (65)	99 (60)	
≥ 10.0		37 (22)	27 (16)	
Gleason Sum				
≤ 6		73 (42)	79 (46)	0.03
7 (3 + 4)		62 (35)	71 (42)	
7 (4 + 3)		25 (14)	9 (5)	
8 -- 10		15 (9)	12 (7)	

Table 2

Odds Ratios for Prostate Cancer Stratified by *TMPRSS2:ERG* Fusion Status in Relation to Current Use of Aspirin, Non-Aspirin NSAIDs, and Acetaminophen

	Controls No. (%)	TMPRSS2/ERG Negative No. (%)	Age-Adjusted OR (95% CI)	Multivariate OR (95% CI) *	TMPRSS2/ERG Positive No. (%)	Age-Adjusted OR (95% CI)	Multivariate OR (95% CI) *
Current Aspirin use							
No	522 (55)	100 (57)	1.00 (referent)	1.00 (referent)	120 (70)	1.00 (referent)	1.00 (referent)
Yes	420 (45)	75 (42)	1.00 (0.71 – 1.41)	0.99 (0.69 – 1.42)	51 (30)	0.68 (0.47 – 1.00)	0.63 (0.43 – 0.93)
Current non-Aspirin NSAID use							
No	815 (87)	151 (86)	1.00 (referent)	1.00 (referent)	155 (91)	1.00 (referent)	1.00 (referent)
Yes	127 (13)	24 (14)	1.01 (0.63 – 1.62)	1.04 (0.64 – 1.67)	16 (9)	0.65 (0.38 – 1.14)	0.65 (0.37 – 1.14)
Current Acetaminophen use							
No	886 (94)	163 (93)	1.00 (referent)	1.00 (referent)	162 (95)	1.00 (referent)	1.00 (referent)
Yes	56 (6)	12 (7)	1.17 (0.21 – 2.24)	1.12 (0.58 – 2.18)	9 (5)	0.94 (0.45 – 1.97)	0.93 (0.43 – 1.98)

*Adjusted for age, race, family history of prostate cancer, prostate cancer screening within 5 years prior to diagnosis (cases) or referent date (controls)

Table 3

Odds Ratios for Prostate Cancer Stratified by *TMPRSS2:ERG* Fusion Status in Relation to Total Duration of Aspirin Use

Duration of Aspirin Use	Controls No (%)	TMPRSS2:ERG Negative No (%)	Age-Adjusted OR (95% CI)	Multivariate OR (95% CI) *	TMPRSS2:ERG Positive No (%)	Age-Adjusted OR (95% CI)	Multivariate OR (95% CI) *
Never user	457 (49)	90 (51)	1.00 (referent)	1.00 (referent)	108 (63)	1.00 (referent)	1.00 (referent)
0.1 – 2.5 years	127 (14)	21 (12)	0.88 (0.52 – 1.47)	0.79 (0.47 – 1.35)	23 (13)	0.90 (0.54 – 1.49)	0.78 (0.46 – 1.31)
2.6 – 4.9 years	90 (10)	14 (8)	0.80 (0.43 – 1.49)	0.87 (0.46 – 1.64)	12 (7)	0.65 (0.34 – 1.26)	0.65 (0.33 – 1.26)
5.0 – 9.9 years	127 (14)	20 (11)	0.84 (0.49 – 1.44)	0.78 (0.45 – 1.36)	16 (9)	0.65 (0.36 – 1.16)	0.54 (0.30 – 0.98)
>= 10 years	139 (15)	30 (17)	1.21 (0.76 – 1.96)	1.19 (0.73 – 1.95)	12 (7)	0.52 (0.27 – 0.99)	0.46 (0.24 – 0.88)

* Adjusted for age, race, family history of prostate cancer, prostate cancer screening within 5 years prior to diagnosis (cases) or referent date (controls)