Title

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Journal

Cancer Epidemiology, Biomarkers & Prevention, 21(9)

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Publication Date

2012-09-01

DOI

10.1158/1055-9965.EPI-12-0042

Peer reviewed

Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2012 September ; 21(9): 1497–1509. doi: 10.1158/1055-9965.EPI-12-0042.

THE *TMPRSS2:ERG* REARRANGEMENT, ERG EXPRESSION, AND PROSTATE CANCER OUTCOMES: A COHORT STUDY AND META-ANALYSIS

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Abstract

Background—Whether the genomic rearrangement *TMPRSS2:ERG* has prognostic value in prostate cancer is unclear.

Methods—Among men with prostate cancer in the prospective Physicians' Health and Health Professionals Follow-Up Studies, we identified rearrangement status by immunohistochemical assessment of ERG protein expression. We used Cox models to examine associations of ERG overexpression with biochemical recurrence and lethal disease (distant metastases or cancer-specific mortality). In a meta-analysis including 47 additional studies, we used random effects models to estimate associations between rearrangement status and outcomes.

Results—The cohort consisted of 1,180 men treated with radical prostatectomy between 1983 and 2005. During a median follow-up of 12.6 years, 266 men experienced recurrence, and 85 men developed lethal disease. We found no significant association between ERG overexpression and biochemical recurrence (HR: 0.99; 95% CI: 0.78-1.26) or lethal disease (HR: 0.93; 95% CI: 0.61-1.43). The meta-analysis of prostatectomy series included 5,074 men followed for biochemical recurrence (1,623 events), and 2,049 men followed for lethal disease (131 events).

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TMPRSS2:ERG was associated with stage at diagnosis ($RR_{\geq T3 \text{ vs. } T2}$: 1.23; 95% CI: 1.16-1.30) but not with biochemical recurrence (RR: 1.00; 95% CI: 0.86-1.17) or lethal disease (RR: 0.99; 95% CI: 0.47-2.09).

Conclusions—These results suggest that *TMPRSS2:ERG*, or ERG overexpression, is associated with tumor stage but does not strongly predict recurrence or mortality among men treated with radical prostatectomy.

Impact—This is the largest prospective cohort study to examine associations of ERG overexpression and lethal prostate cancer among men treated with radical prostatectomy.

Keywords

TMPRSS2:ERG; prostate cancer; gene fusion; biomarker; prognosis

INTRODUCTION

In 2005, Tomlins and colleagues identified the *TMPRSS2:ERG* gene fusion as a common genetic event in prostate cancer (1). Their finding is notable in that recurrent chromosomal rearrangements were previously observed primarily in hematologic cancers and tumors of mesenchymal origin (2). An estimated 40 to 50% of prostate cancers harbor the fusion (3), translating to approximately 100,000 new cases of fusion positive prostate cancer in the United States each year (4).

The gene fusion involves *TMPRSS2* (transmembrane protease, serine 2) and *ERG* (v-ets erythroblastosis virus E26 oncogene homolog), both located on chromosome 21. Their fusion can occur as a result of either a chromosomal translocation or an interstitial deletion (5, 6). The *TMPRSS2* gene is androgen regulated and the oncogene *ERG* is a member of the erythroblast transformation specific (ETS) family of transcription factors (7), which plays a role in the regulation of proliferation, differentiation, apoptosis, and other cellular processes (8, 9). The gene fusion may thus reflect a mechanism of androgen regulation of downstream oncogenic effects that could influence prostate cancer progression.

Given the potential significance of *TMPRSS2:ERG*, several studies have investigated whether or not patients with prostate tumors exhibiting the fusion are more likely to have cancers with aggressive pathologic characteristics or to experience disease progression. Some studies have found a positive association between *TMPRSS2:ERG* and prostate cancer progression (6, 10-14), while other studies have observed null, or inverse, associations between fusion status and poor outcomes (15-26). Results from studies examining the association between *TMPRSS2:ERG* and clinicopathologic features, such as tumor stage and Gleason grade, are also mixed (6, 12-14, 16-23, 25, 27-33). The difference in findings is likely explained in part by the small sample sizes and limited number of events in most prior studies, as well as by heterogeneity of study cohorts (e.g., radical prostatectomy versus watchful waiting cohorts), tumor tissue assessed for the fusion (e.g., tissue from radical prostatectomy specimens versus tissue from transurethral resections of the prostate (TURPs)), and technique used to detect the fusion (e.g., fluorescence in situ hybridization (FISH) versus reverse transcription polymerase chain reaction (RT-PCR)).

The aim of the current study was to investigate whether the *TMPRSS2:ERG* fusion is associated with a more aggressive phenotype of prostate cancer and ultimately worse prognosis. We first conducted a prospective cohort study assessing the association between ERG protein overexpression (a marker of the fusion) and clinicopathologic factors, as well as recurrence and prostate cancer mortality among 1,180 men treated with radical prostatectomy. We then compared our results with previous studies via a systematic meta-

analysis of prior research on the association between *TMPRSS2:ERG* and clinicopathologic factors and progression.

MATERIALS AND METHODS

COHORT STUDY

Study population—The study was nested among US men diagnosed with prostate cancer who were participants in the Physicians' Health Study I and II and the Health Professionals Follow-Up Study. The Physicians' Health Study I was a randomized trial of aspirin and beta-carotene among 22,071 male physicians aged 40-84 at randomization in 1982 (34). From 1995 to 1997, 7,641 participants from the Physicians' Health Study I were enrolled in the Physicians' Health Study II, a randomized trial of vitamin use (clinicaltrials.gov Identifier: NCT00270647) (35). Other Physicians' Health Study I participants continued follow-up via parallel annual questionnaires. The Health Professionals Follow-up Study is an ongoing prospective study of causes of cancer and other diseases among 51,529 male health professionals aged 40-75 at enrollment in 1986. Men in both studies were free of diagnosed cancer, excluding non-melanoma skin cancer, at baseline.

Clinical and follow-up data of men with prostate cancer—Prostate cancer diagnoses were initially identified by self-report, and then confirmed by review of medical records and pathology reports. The study team also reviewed medical records to abstract information on tumor stage, prostate specific antigen (PSA) level at diagnosis, and treatments. Since 2000, participants with prostate cancer have been followed for biochemical recurrence and development of metastases via questionnaires. For men with prostate cancer in the Health Professionals Follow-up Study, the patients' treating physicians were also contacted to collect information about clinical course, including confirmation of the development of metastases. For men with prostate cancer in the Physicians' Health Study, we were able to verify reports of metastases in approximately 80% of the cases by reviewing medical records. Biochemical recurrence was either participant reported, reported by the treating physician, or abstracted from medical records. When abstracted from medical records, it was defined as PSA above 0.2 ng/mL post-surgery sustained over two measures. The date of first rise in PSA was considered the date of biochemical recurrence. Study physicians assigned cause of death following a centralized review of medical records and death certificates. Prostate cancer was defined as the cause of death when there was evidence of extensive metastatic disease, and when there was no other more plausible cause of death. Follow-up for mortality in the cohorts is greater than 95%.

Tumor tissue cohort—Among men in both the Physicians' Health Study and Health Professionals Follow-up Study, we sought to retrieve archival tumor tissue materials for men who underwent radical prostatectomy or TURP. The present study included formalin-fixed paraffin-embedded tumor specimens from 1,292 men with prostate cancer: 443 in the Physicians' Health Study diagnosed between 1982 and 2004, and 849 in the Health Professionals Follow-up Study diagnosed between 1986 and 2005.

The study pathologists (R.T.L., R.F., M.F., M.L.) reviewed hematoxylin and eosin slides to provide uniform Gleason grade and other histopathological features, and to select areas of tumor for construction of tumor tissue microarrays (36). Tissue microarrays were constructed by taking at least three 0.6-mm cores of tumor tissue per case from the primary tumor nodule or the nodule with the highest Gleason grade and transferring to a recipient block. The tumor specimens from the 1,292 cases were included on twelve tissue microarrays.

Assessment of ERG status by immunohistochemistry—We characterized presence or absence of *TMPRSS2:ERG* in tumors included on the tissue microarrays by immunohistochemical evaluation of ERG protein expression, which has previously been shown to have high concordance with *TMPRSS2:ERG* fusion status as assessed by FISH (37, 38) and quantitative PCR (39). We used a BioGenex i6000 automated staining platform (BioGenex Laboratories Inc., Fremont CA). Five-μm formalin-fixed, paraffin-embedded sections of each tissue microarray were deparaffinized in xylene, followed by a graded alcohol rehydration. Antigen retrieval was performed by microwaving the tissue in citrate buffer for 5 minutes. ERG antisera (Clone ID: EPR3864, Epitomics, Inc., Burlingame, CA) was applied at 1:100 for 1 hour. Detection of the primary ERG antibody was carried out using the BioGenex SS Multilink secondary antibody, followed by horseradish peroxidase (HRP) conjugation to the secondary antibody using the Biogenex SS HRP Labeling kit. Visualization of ERG was accomplished using the DAB substrate kit (Vector Laboratories Inc., Burlingame, CA). Sections were subsequently counterstained with hematoxylin, and the sections were dehydrated in a graded series of alcohol prior to coverslip application.

Tumor specimens were analyzed for ERG expression by a study pathologist (R.T.L.). For all cases, the presence of ERG staining in the vasculature endothelium served as a positive internal control, and subsequent assessment of ERG was restricted to cores in which the positive internal control was observed. A case was called positive for ERG expression (i.e. ERG overexpression) if at least one core from an individual case had positive ERG staining observed within prostate cancer epithelial cells. Of cases positive for ERG on at least one core, 85% stained positive for ERG in all cores evaluated. When ERG status could not be assessed due to lack of remaining tumor tissue or negative internal endothelial control (n=121), sections of the original tumor blocks were re-stained for ERG.

Statistical analysis—We excluded from the statistical analyses men with unknown ERG status due to lack of remaining tumor tissue or men whose internal endothelial control stained negative (n=22). Of the remaining 1,270 men (433 men from the Physicians' Health Study, and 837 men from the Health Professionals Follow-up Study), 1,180 had undergone radical prostatectomy and 90 had undergone TURP. As the association between *TMPRSS2:ERG* and disease progression may differ depending on tumor tissue assessed for the fusion and/or primary treatment received, our primary analyses focused on men who had undergone radical prostatectomy; data on the TURP cases are presented in the supplemental materials (Supplemental Table 1).

We investigated whether or not age at diagnosis and follow-up time differed by ERG overexpression status using a *t*-test. For categorical analyses, we used Chi-Square tests or Cochrane-Armitage trend tests to look for differences by ERG overexpression status across categories of pathological tumor stage, Gleason score, and PSA level at diagnosis.

To investigate the association between ERG overexpression and disease progression, we used time to event analyses and Cox proportional hazards models to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). We defined prostate cancer progression in two ways: 1) time to lethal prostate cancer, defined as development of distant metastases or prostate cancer death, and 2) time to biochemical recurrence. Men who did not report a PSA rise but who reported lymph node metastases, distant metastases, or who died of prostate cancer were assigned a biochemical recurrence on the earliest date of any of these events. Men in the cohort were followed from the date of prostate cancer diagnosis until they experienced outcomes, until they were censored at death from other causes, or at end of follow-up, whichever occurred first. Follow-up for death ended in March 2011 for men in the Physicians' Health Study, and May 2011 for men in the Health Professionals Follow-up Study. In both cohorts, follow-up for recurrence and metastases ended approximately one

year before follow-up for death due to questionnaire timing. Men with missing information on pathological tumor stage (n=38) were assigned their clinical tumor stage when available (n=33), or to the reference category of T2 tumors (n=5) in the multivariate models. We also ran multivariate models restricted to men with known pathological tumor stage (n=1,142). Men diagnosed with prostate cancer before the onset of PSA testing or with unknown PSA level at diagnosis were assigned a missing indicator variable (n=114).

All analyses were conducted using SAS version 9.2 (SAS institute Inc., Cary, NC, US). All tests were 2-sided with p-values <0.05 considered statistically significant. This part of the study was approved by the institutional review boards at the Harvard School of Public Health and Partners Health Care.

META-ANALYSIS

Identification and eligibility of relevant studies—We sought to include all cohort and cross-sectional studies published in English addressing the associations between the *TMPRSS2:ERG* fusion and five distinct prostate cancer outcomes: tumor stage (pathological stage for radical prostatectomy cohorts, clinical stage otherwise), Gleason score (from radical prostatectomy, biopsy or TURP), biochemical recurrence, lethal prostate cancer (prostate cancer specific death and distant metastases), and age at diagnosis. In June 2010, we searched literature available from PubMed, Embase, Medline, and BIOSIS published after October 2005 (at which point Tomlins and colleagues published their paper (1)), and we performed an updated search in April 2012. The search strategy included a combination of the terms *prostate* or *prostatic*, *cancer(s)* or *neoplasm(s)*, and *TMPRSS2* (or *ERG* in April 2012 only). We screened 884 abstracts and full articles lacking abstracts to assess relevance and reviewed 126 full article texts for selected studies. Upon study selection, we further reviewed the reference section of each article to identify additional relevant studies. Data were extracted directly from the published papers or else, when the data were not available in the appropriate format, we attempted to collect the data via contact with relevant investigators. We contacted corresponding authors for 34 papers discovered during the June 2010 search, as well as Minner and colleagues (because of the study's large sample size) (26), and received additional data for sixteen.

We excluded studies for which the assay to identify fusion status did not differentiate between positive and negative tumors (but rather reported results on *TMPRSS2:ERG* expression continuously) (n=2) (40, 41). We also omitted studies in which no outcomes of interest occurred (n=1) (42). Studies containing inconsistencies in the data presented in the paper were excluded as well (n=2) (43, 44), as were cohorts looking at certain subsets of prostate cancer (n=5) (45-49). We excluded one further study for which we could not determine the procedure used to collect tumor tissue (n=1) (50). In the case of multiple articles with possible overlapping data, we selected studies with the most complete data.

Data extraction—Using a standardized data extraction template, two investigators (A.P., R.E.G.) independently extracted and tabulated the relevant data. They reached a consensus on any inconsistencies in data extraction by examination of the original articles and discussion. For articles reporting results on multiple patient subgroups defined by different criteria (e.g., fusions assayed in radical prostatectomy specimens and, separately, fusions assayed in TURP specimens), we considered each subgroup as a separate cohort for extraction and statistical analysis. Given that both *TMPRSS2* and *ERG* most commonly fuse with each other (3), we considered a case to be fusion positive whenever an assay indicated *TMPRSS2*, *ERG*, or *TMPRSS2:ERG* rearrangement, or overexpression of the *TMPRSS2:ERG* gene fusion (mRNA) or ERG protein. Information extracted included cohort country, study time period, treatment strategies, assay used to assess fusion status,

tissue assayed for the fusion, population size, average population age, number of patients within each fusion category (negative, positive, translocation, and deletion) and outcomes data.

Statistical analysis—We calculated unadjusted risk ratios (RRs) as the measure of association between fusion status and tumor stage (T3 or greater compared to T2 or less), Gleason score (Gleason 8 to 10 compared to Gleason 2 to 6 in order to compare the extremes), biochemical recurrence (yes/no), and lethal prostate cancer (yes/no). As a secondary analysis, we also assessed the association between fusion status and risk of Gleason 7 versus Gleason 2 to 6 tumors among all men and then restricted to those treated by radical prostatectomy. For cohorts in which one exposure group did not have at least one event in both outcome categories, we added a 0.05 continuity correction to all cell counts. RR measures were natural log transformed and pooled via DerSimonian and Laird random effects models (51). For age at diagnosis, we calculated weighted mean differences (WMDs) as the measure of association with fusion status. Age analyses were restricted to the subset of studies for which standard deviations of the means in each exposure group were available. (Ten cohorts had mean ages in each exposure group but did not have standard deviations available.) For each outcome, except for tumor stage, we first compared positive versus negative fusion status among all patients. For tumor stage, we only present results stratified by tissue assayed (radical prostatectomy, TURP, biopsy, lymph nodes, urine) so as to separate associations of ERG status with pathological stage (radical prostatectomy cohorts only) and clinical stage (all other tissue categories). We also conducted analyses by tissue assayed for Gleason score and age at diagnosis. For recurrence and lethal prostate cancer, we conducted analyses by primary treatment (radical prostatectomy, watchful waiting, hormones, radiation therapy, or other).

We assessed each association for potential effect modification by average cohort length of follow-up (applicable for only recurrence and lethal prostate cancer) and average cohort age at diagnosis where possible. We conducted analyses comparing patients positive-by-translocation versus those negative for the fusion, and positive-by-deletion versus those negative for the fusion, when data were available. We also conducted a subgroup analysis by assay used to assess fusion status (FISH, RT-PCR (including one study using a TaqMan Low-Density Array) (52), immunohistochemistry (IHC)), using only studies having assayed radical prostatectomy specimens to avoid differences by tissue type.

All statistical analyses for the meta-analysis were conducted using STATA/IC 10.0 for Mac (STATACorp, College Station, Tex). All tests were 2-sided with p-values <0.05 considered statistically significant.

RESULTS

COHORT STUDY

The radical prostatectomy cohort of men from the Physicians' Health Study and Health Professionals Follow-up Study consisted of 1,180 men diagnosed with prostate cancer between 1983 and 2005. Mean age at diagnosis was 65.4 years (range 47 to 86). Most men had pT2 (72%) and pathological Gleason score 3+4 (37%) tumors (Table 1). During a median follow-up of 12.6 years, 266 men experienced biochemical recurrence. Sixty-three men died of prostate cancer and an additional 22 men were diagnosed with metastases to distant organs. In total, 311 men died of any cause during follow-up.

Forty-nine percent of men in the prostatectomy cohort had tumors overexpressing ERG (Table 1). Men whose tumors overexpressed ERG were more likely to be diagnosed at a higher tumor stage (p-value: <0.01); the prevalence of tumors overexpressing ERG was 65%

among men with pT4/N1/M1 tumors compared to 47% among men with pT2 tumors. We found no association between ERG overexpression and Gleason score (p-value: 0.58), or with age at diagnosis (p-value: 0.17). However, ERG overexpression was associated with lower PSA level at diagnosis (p-value: 0.02), and associated with a slightly longer mean follow-up time (13.0 versus 12.2 years) (p-value: <0.01). Among men diagnosed before 1992 (pre-PSA era) the prevalence of ERG overexpression was 54%, whereas it was 49% among men diagnosed after 1992 (PSA era).

We found no association between ERG overexpression and risk of lethal prostate cancer post-prostatectomy in either the age and cohort adjusted model or the multivariate model (Table 2). The HR was 0.93 (95% CI: 0.61-1.43) in the model adjusted for age at diagnosis and cohort. Additional adjustment for Gleason score and tumor stage did not qualitatively change the risk estimate (HR: 0.85; 95% CI: 0.55-1.31). Similarly, there was no association between ERG overexpression and biochemical recurrence or all cause mortality. Additional adjustment for PSA level at diagnosis and restriction of the analyses to men with known pathological tumor stage did not materially change any of the risk estimates (data not shown).

META-ANALYSIS

Supplemental Figure 1 summarizes the study selection process for the systematic literature search, which resulted in 48 (6, 10-23, 25-33, 39, 52-73) studies (including our cohort study), 62 total cohorts and 10,803 subjects used in the analyses. Characteristics of the cohorts and patients included in the studies are presented in Supplemental Table 2. Studies that were reviewed, but excluded, are described in Supplemental Table 3. When we excluded our cohort from the meta-analysis, estimates did not, with few exceptions (noted at the end of the Results section), materially change (data not shown). All subsequent results refer to those from the meta-analysis including our cohort.

The prevalence of the fusion across all of the cohorts was 47% (Table 3). In radical prostatectomy samples, the prevalence of the fusion was higher among patients assayed by RT-PCR (52%) and IHC (52%) relative to those assayed by FISH (42%). Prevalence was lower in Asian cohorts (23%) than in European (54%) and North American (48%) cohorts. The prevalence of the fusion in tumors from prostate cancer patients for whom a TURP specimen was analyzed was 30%. Twenty-four cohorts examined the mechanism through which the *TMPRSS2:ERG* gene fusion occurred; 64% of patients with tumors that harbor the fusion were positive by deletion. The proportion of fusion positive tumors that occurred by deletion was fairly consistent across populations and by type of tissue specimen analyzed (Table 3).

Figures 1A – D present the relative risk estimates from 61 cohorts for the association between the *TMPRSS2:ERG* fusion and the prostate cancer outcomes. Men with fusion positive cancers were somewhat more likely to have advanced stage tumors (T3 or greater versus T2 or lower) (Figure 1A). Within subgroups defined by tissue assayed, studies showed no significant heterogeneity for associations between fusion status and stage. Fusion status was not associated with risk of Gleason 8 to 10 versus 2 to 6 prostate cancer (RR: 0.99; 95% CI: 0.86-1.13) or Gleason 7 versus 2 to 6 disease (RR: 1.05; 95% CI: 0.99-1.12). Figures 1C and D present the associations between fusion status and biochemical recurrence (RR: 1.02; 95% CI: 0.89-1.16) and lethal prostate cancer (RR: 1.12; 95% CI: 0.83-1.51). For all of these endpoints, the I-squared test indicated significant between-study heterogeneity. Continuous average length of follow-up did not significantly modify associations between fusion status and biochemical recurrence or lethal prostate cancer (data not shown). With the exception of the association between fusion status and Gleason 8 to 10 versus 2 to 6 at diagnosis (p-value: 0.04), continuous average age at diagnosis did not modify any

associations between fusion status and outcomes (data not shown). Among cohorts of men for which the average age of diagnosis was below the median of 63.8, the risk ratio comparing Gleason 8 to 10 versus 2 to 6 tumors was 0.83 (95% CI: 0.64-1.08). Among those above the median average age, the risk ratio was 1.07 (95% CI: 0.89-1.30).

With regard to age at diagnosis, the pooled results suggested that patients whose tumors were positive for the fusion were diagnosed at slightly younger ages than those negative for the fusion, (WMD: −0.89 years; 95% CI: −1.46 - −0.31). The studies showed significant heterogeneity for associations with mean age at diagnosis (I^2: 40.2%; p-value: 0.03).

Among men treated with radical prostatectomy, the meta-analysis indicated a positive association between fusion status and higher stage at diagnosis (RR: 1.23; 95% CI: 1.16-1.30). Fusion status, however, was not associated with Gleason score; the risk ratio comparing Gleason 8 to 10 versus 2 to 6 tumors was 0.85 (95% CI: 0.72-1.01) and that comparing Gleason 7 versus 2 to 6 tumors was 1.03 (95% CI: 0.97-1.09). Among those treated with radical prostatectomy, the meta-analysis included 5,074 men who were followed for biochemical recurrence (1,623 events), and 2,049 men who were followed for distant metastases and prostate cancer death (131 events). In line with the results from our cohort study, the associations between fusion status and biochemical recurrence and lethal prostate cancer respectively were null (Figures 1C and D). Results for age were also consistent with the overall analysis (WMD: −0.66 years; 95% CI: −1.34-0.02).

Analyses of fusion status in patients diagnosed by TURP were slightly more suggestive of associations with poor outcomes. Men whose tumors were fusion positive were well over two times as likely to be diagnosed with cancers at a higher clinical stage (RR: 2.65; 95% CI: 1.72-4.09). Results for Gleason score at diagnosis were also suggestive of an association (RR: 1.61; 95% CI: 1.01-2.57). The meta-analysis included 227 men diagnosed with TURP who were followed for distant metastases and prostate cancer death (84 events). Though not statistically significant, men with fusion positive tumors who were diagnosed by TURP were 1.37 (95% CI: 0.53-3.51) times as likely to experience distant metastases or die from prostate cancer as those negative for the fusion (Figure 1D). This finding is consistent with our cohort of 90 men diagnosed by TURP (Supplemental Table 1). Results for mean age at diagnosis were null (WMD: −0.16, 95% CI: −1.12-0.79).

Data on outcomes broken out by mechanism of the fusion, (e.g., positive by deletion versus fusion negative), were available in 17 cohorts. There was no difference in the risk of advanced pathological stage, high grade, biochemical recurrence, lethal prostate cancer, or age at diagnosis comparing patients with tumors whose fusion arose by translocation or deletion versus those negative for the fusion (Supplemental Table 4).

Among men treated with radical prostatectomy, the associations did not vary significantly by the assay used to determine fusion status (Supplemental Table 5). Some of these results, however, were based on data from very few studies.

Upon running all analyses excluding data from our cohort study, few results materially changed. Among men diagnosed by TURP, the association between fusion status and Gleason score at diagnosis changed from 1.61 (95% CI: 1.01-2.57) to 1.66 (95% CI: 0.94, 2.93). Age at diagnosis also no longer modified the association between fusion status and Gleason 8 to 10 versus 2 to 6 prostate cancer (p-value: 0.06). Our cohort also provided the only data for analyses of the associations between fusion status and mortality among those assayed by IHC.

DISCUSSION

Since its discovery in 2005, the common gene fusion *TMPRSS2:ERG* has been extensively studied as a possible biomarker for prostate cancer progression with mixed results (11, 12, 15, 16, 19-23, 26, 27, 54, 57-59, 62-65). We analyzed data from the largest cohort study with lethal disease as an endpoint, and found no association between ERG overexpression and risk of lethal prostate cancer. The meta-analysis yielded similarly null results for the analysis of fusion status and biochemical recurrence. These findings suggest that the *TMPRSS2:ERG* fusion is not a strong predictive marker of disease outcome among men with prostate cancer treated with radical prostatectomy.

Among men treated with radical prostatectomy, both in the cohort study and in the meta-analysis, ERG overexpression or positive *TMPRSS2:ERG* fusion status was associated with a more advanced tumor stage. Among these men, the positive association with stage but not with recurrence or death from prostate cancer would be possible if presence of the fusion in prostate tumors were associated with local tumor growth rather than metastatic spread. Then in men treated with radical prostatectomy, who are most often treated before the tumor has spread beyond the prostate capsule, the fusion would not predict outcome.

The prevalence of *TMPRSS2:ERG* varied according to several factors. In the meta-analysis, the prevalence of the fusion was 49% in tissue from radical prostatectomy samples and only 30% in tissue from TURP samples. This difference may reflect previous findings that the fusion is less common in transition zone tumors (from which most tumors found in TURP samples presumably originate) (55, 74) than in peripheral zone tumors (55, 59, 71, 75, 76). It also supports the notion that prostate cancers originating from the two zones may be genetically or biologically distinct (55, 59). The prevalence of the fusion further differed by continent of the patient cohort; it was 23% in Asian cohorts, and roughly 50% in European and North American cohorts. Several factors could explain these differences, including varying distributions of genetic or lifestyle factors associated with the risk of developing fusion negative versus positive prostate cancer. We also found that in radical prostatectomy samples the prevalence of the fusion was higher in studies using RT-PCR (52%) or IHC (52%) to assess *TMPRSS2:ERG* fusion status, relative to studies using FISH (42%). The results indicate that some, if not all, methods may misclassify *TMPRSS2:ERG* fusion status to some degree.

Findings from some previous studies suggest that *TMPRSS2:ERG* may be associated with worse outcomes among patients managed with watchful waiting following a diagnosis of prostate cancer by TURP (10, 11). Demichelis, Fall, and colleagues found an almost three-fold increased risk of distant metastases and prostate cancer death among men who were fusion positive versus negative (11). This finding was later replicated in a large case-control study by the same group using an “extreme” case design of lethal and indolent prostate cancers (77). In another TURP cohort, Attard and colleagues found that men who harbor a fusion occurring by deletion specifically were at an increased risk of death compared to men with fusion negative prostate cancer (10). Only three TURP cohorts (including 90 men from our cohort study) were available for our analysis investigating the association between *TMPRSS2:ERG* and lethal prostate cancer. There were similarly few studies providing data on tumor grade and stage in this patient group. Even so, the results align with the hypothesis that *TMPRSS2:ERG* occurs at a lower frequency but is associated with a more aggressive phenotype in patients undergoing watchful waiting after TURP. It is possible that *TMPRSS2:ERG* is associated with progression in this particular patient group because tumors occurring in the transition zone are biologically or genetically different from tumors occurring in the peripheral zone. It is also possible, given that peripheral zone tumors have been associated with poorer prognosis compared to transition zone tumors (78), that positive

fusion status in TURP samples is simply a marker of peripheral zone tumor origin for that subset of cancers. Yet another explanation is that *TMPRSS2:ERG* indeed is a prognostic marker in prostate cancer. If *TMPRSS2:ERG* is a marker of local tumor growth rather than metastatic spread, in the absence of therapy, men with fusion positive tumors should have a poorer prognosis than men with tumors not harboring the fusion. A large-scale biopsy study among men with and without initial therapy is needed to answer this question.

It has been suggested that cancers harboring gene fusions occurring by deletion have worse prognosis than those occurring by translocation, possibly because the ~3 Mb between *TMPRSS2* and *ERG* on chromosome 21 contain influential tumor suppressor genes (6). *TMPRSS2:ERG* fusions occurring through deletion would thus be coupled with down-regulation of tumor suppressor genes as well as up-regulation of an oncogene. Our meta-analysis did not support this hypothesis. We did not find significant associations between *TMPRSS2:ERG* positive by translocation or positive by deletion cancers (both versus *TMPRSS2:ERG* negative cancers) and outcomes. These results should be interpreted with caution, however, as the number of studies for which we had data in the appropriate format limited our analyses.

It is possible that our cohort study was limited by the use of an indirect method, immunohistochemical expression of ERG, to assess fusion status. A small proportion (~10%) of tumors that overexpress ERG may harbor a fusion between *ERG* and genes other than *TMPRSS2*, including *SLC45A3* or *NDRG1*. If it is *TMPRSS2:ERG* specifically rather than ERG overexpression that is associated with prostate cancer progression, this exposure misclassification would likely have led to attenuation of the risk estimates. The meta-analysis, however, yielded similar results among those assayed by FISH and RT-PCR, indicating that our use of an IHC assay unlikely accounted for our null findings. Another limitation is that we could not examine whether specific subtypes of the fusion are associated with progression. For example, prior studies have suggested that certain fusion transcript variants (13, 27, 79) and increased copy number of the rearrangement (10, 14, 15, 57) are associated with outcomes. We did not address some additional important questions, including whether or not other ETS fusion partners of *TMPRSS2*, among them *ETV1, ETV4*, and *ETV5,* are associated with prostate cancer progression.

The meta-analysis was limited by the data available in the appropriate format; there were some studies that reported on relevant endpoints from which we were unable to acquire data eligible for our analyses. Importantly, the meta-analysis was limited by the small number of studies examining lethal prostate cancer. The results from these studies were furthermore highly heterogeneous, as reflected by the wide confidence interval of the pooled risk ratio. More studies examining lethal prostate cancer are needed. The meta-analysis also included few studies on the risk of prostate cancer progression following hormonal treatment, radiation therapy, or chemotherapy, subgroups in which *TMPRSS2:ERG* may be a predictive marker (80, 81).

This study has important strengths. Our analysis includes the largest prospective prostatectomy cohort examining the association between *TMPRSS2:ERG* and lethal prostate cancer published to-date (80% power to detect a relative risk of 1.5 assuming a 10% risk of lethal disease among the unexposed). This is important since biochemical recurrence is an imprecise predictor of prostate cancer death (82, 83). The meta-analysis supplied increased power primarily to analyses of *TMPRSS2:ERG* status in relation to tumor stage, Gleason score, biochemical recurrence, and mean age at diagnosis. That the results from the cohort study and meta-analysis were similar among men treated with radical prostatectomy reinforces the validity of the findings in the cohort study. In addition, one large nested case-

control study ineligible for the meta-analysis also found no significant association between fusion status and biochemical recurrence or clinical progression (24).

In summary, the results from this cohort study and meta-analysis suggest that among men undergoing radical prostatectomy, *TMPRSS2:ERG* fusion status is not a strong predictor of prostate cancer recurrence or cancer-specific mortality. It is at the same time clear that the role of *TMPRSS2:ERG* in prostate cancer pathogenesis and progression is only starting to emerge. Particular subtypes of the fusion, fusion status in specific subgroups of patients, and interaction of the fusion with other factors such as specific genetic events or treatment regimens could ultimately prove important for treatment choices and prognosis. Notably, whether or not the *TMPRSS2:ERG* fusion is a prognostic marker in men with prostate cancer left untreated, or if the fusion is a predictive marker of outcome among men treated with radiation or chemotherapy, are important questions that remain largely unstudied and unanswered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the participants and staff of the Physicians' Health Study and Health Professionals Follow-up Study for their valuable contributions. In addition we would like to thanks the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The Dana-Farber/Harvard Cancer Center Tissue Microarray Core Facility constructed the tissue microarrays in this project. We thank the librarians at the Countway Library of Medicine for providing support on the literature search, and we thank all investigators who provided us with raw data from their studies or were in other ways helpful. We also thank Jennifer A. Sinnott for her valuable help with the statistical analysis.

GRANT SUPPORT This work was supported by the Dana-Farber / Harvard Cancer Center Specialized Programs of Research Excellence program in prostate cancer (5P50CA090381-08); and the National Cancer Institute (T32 CA009001 to K.L.P., CA55075, CA141298, CA13389, CA-34944, CA-40360, CA-097193, EDRN U01 CA113913 and PO1 CA055075); and the National Heart, Lung, and Blood Institute (HL-26490 and HL-34595); and the Prostate Cancer Foundation (to L.A.M. and N.E.M.); and the National Institute of Health (R25 CA098566 to R.E.G.); and the Swedish Research Council (Reg. No. 2009-7309 to A.P.); and the Birgit and Hellmuth Hertz' Foundation (to A.P.)

REFERENCES

1. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310:644–8. [PubMed: 16254181]
2. Frohling S, Dohner H. Chromosomal abnormalities in cancer. N Engl J Med. 2008; 359:722–34. [PubMed: 18703475]
3. Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol. 2009; 56:275–86. [PubMed: 19409690]
4. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61:212–36. [PubMed: 21685461]
5. Hermans KG, van Marion R, van Dekken H, Jenster G, van Weerden WM, Trapman J. TMPRSS2:ERG fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptor-negative prostate cancer. Cancer Res. 2006; 66:10658–63. [PubMed: 17108102]

6. Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, et al. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res. 2006; 66:8337–41. [PubMed: 16951139]
7. Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. Cancer Res. 1999; 59:4180–4. [PubMed: 10485450]
8. Seth A, Watson DK. ETS transcription factors and their emerging roles in human cancer. Eur J Cancer. 2005; 41:2462–78. [PubMed: 16213704]
9. Clark JP, Cooper CS. ETS gene fusions in prostate cancer. Nat Rev Urol. 2009; 6:429–39. [PubMed: 19657377]
10. Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, et al. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene. 2008; 27:253–63. [PubMed: 17637754]
11. Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene. 2007; 26:4596–9. [PubMed: 17237811]
12. Nam RK, Sugar L, Yang W, Srivastava S, Klotz LH, Yang LY, et al. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer. 2007; 97:1690–5. [PubMed: 17971772]
13. Wang J, Cai Y, Ren C, Ittmann M. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res. 2006; 66:8347–51. [PubMed: 16951141]
14. Yoshimoto M, Joshua AM, Cunha IW, Coudry RA, Fonseca FP, Ludkovski O, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol. 2008; 21:1451–60. [PubMed: 18500259]
15. Gopalan A, Leversha MA, Satagopan JM, Zhou Q, Al-Ahmadie HA, Fine SW, et al. TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer Res. 2009; 69:1400–6. [PubMed: 19190343]
16. Lapointe J, Kim YH, Miller MA, Li C, Kaygusuz G, Van De Rijn M, et al. A variant TMPRSS2 isoform and ERG fusion product in prostate cancer with implications for molecular diagnosis. Mod Pathol. 2007; 20:467–73. [PubMed: 17334351]
17. Rostad K, Hellwinkel OJ, Haukaas SA, Halvorsen OJ, Oyan AM, Haese A, et al. TMPRSS2:ERG fusion transcripts in urine from prostate cancer patients correlate with a less favorable prognosis. APMIS. 2009; 117:575–82. [PubMed: 19664128]
18. Hofer MD, Kuefer R, Maier C, Herkommer K, Perner S, Demichelis F, et al. Genome-wide linkage analysis of TMPRSS2-ERG fusion in familial prostate cancer. Cancer Res. 2009; 69:640–6. [PubMed: 19147579]
19. Lee K, Chae JY, Kwak C, Ku JH, Moon KC. TMPRSS2-ERG gene fusion and clinicopathologic characteristics of Korean prostate cancer patients. Urology. 2010; 76:1268–e13. [PubMed: 20800881]
20. Leinonen KA, Tolonen TT, Bracken H, Stenman UH, Tammela TLJ, Saramaki OR, et al. Association of SPINK1 expression and TMPRSS2:ERG fusion with prognosis in endocrine-treated prostate cancer. Clin Cancer Res. 2010; 16:2845–51. [PubMed: 20442300]
21. Rouzier C, Haudebourg J, Carpentier X, Valerio L, Amiel J, Michiels JF, et al. Detection of the TMPRSS2-ETS fusion gene in prostate carcinomas: retrospective analysis of 55 formalin-fixed and paraffin-embedded samples with clinical data. Cancer Genet Cytogenet. 2008; 183:21–7. [PubMed: 18474293]
22. Rubio-Briones J, Fernandez-Serra A, Calatrava A, Garcia-Casado Z, Rubio L, Bonillo MA, et al. Clinical implications of TMPRSS2-ERG gene fusion expression in patients with prostate cancer treated with radical prostatectomy. J Urol. 2010; 183:2054–61. [PubMed: 20303538]
23. Saramaki OR, Harjula AE, Martikainen PM, Lvessella R, Tammela TLJ, Visakorpi T. TMPRSS2. ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. Clin Cancer Res. 2008; 14:3395–400.

24. Toubaji A, Albadine R, Meeker AK, Isaacs WB, Lotan T, Haffner MC, et al. Increased gene copy number of ERG on chromosome 21 but not TMPRSS2-ERG fusion predicts outcome in prostatic adenocarcinomas. Mod Pathol. 2011; 24:1511–20. [PubMed: 21743434]
25. Darnel AD, Lafargue CJ, Vollmer RT, Corcos J, Bismar TA. TMPRSS2-ERG fusion is frequently observed in Gleason pattern 3 prostate cancer in a Canadian cohort. Cancer Biol Ther. 2009; 8:125–30. [PubMed: 19029822]
26. Minner S, Enodien M, Sirma H, Luebke AM, Krohn A, Mayer PS, et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of anti-hormonal therapy. Clin Cancer Res. 2011; 17:5878–88. [PubMed: 21791629]
27. Hermans KG, Boormans JL, Gasi D, Van Leenders GJHL, Jenster G, Verhagen PCMS, et al. Overexpression of prostate-specific TMPRSS2(exon 0)-ERG fusion transcripts corresponds with favorable prognosis of prostate cancer. Clin Cancer Res. 2009; 15:6398–403. [PubMed: 19825963]
28. Mao C, Yu Y, Boyd LK, Ren G, Lin D, Chaplin T, et al. Distinct genomic alterations in prostate cancers in Chinese and western populations suggest alternative pathways of prostate carcinogenesis. Cancer Res. 2010; 70:5207–12. [PubMed: 20516122]
29. Miyagi Y, Sasaki T, Fujinami K, Sano J, Senga Y, Miura T, et al. ETS family-associated gene fusions in Japanese prostate cancer: analysis of 194 radical prostatectomy samples. Mod Pathol. 2010; 23:1492–8. [PubMed: 20693979]
30. Mosquera JM, Mehra R, Regan MM, Perner S, Genega EM, Bueti G, et al. Prevalence of TMPRSS2-ERG fusion prostate cancer among men undergoing prostate biopsy in the United States. Clin Cancer Res. 2009; 15:4706–11. [PubMed: 19584163]
31. Sun QP, Li LY, Chen Z, Pang J, Yang WJ, Zhou XF, et al. Detection of TMPRSS2-ETS fusions by a multiprobe fluorescence in situ hybridization assay for the early diagnosis of prostate cancer: a pilot study. J Mol Diagn. 2010; 12:718–24. [PubMed: 20616363]
32. Tu JJ, Rohan S, Kao J, Kitabayashi N, Mathew S, Chen Y-T. Gene fusions between TMPRSS2 and ETS family genes in prostate cancer: frequency and transcript variant analysis by RT-PCR and FISH on paraffin-embedded tissues. Mod Pathol. 2007; 20:921–8. [PubMed: 17632455]
33. Magi-Galluzzi C, Tsusuki T, Elson P, Simmerman K, Lafargue C, Esgueva R, et al. TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. Prostate. 2011; 71:489–97. [PubMed: 20878952]
34. Hennekens CH, Eberlein K. A randomized trial of aspirin and beta-carotene among U.S. physicians. Prev Med. 1985; 14:165–8. [PubMed: 3900975]
35. Gaziano JM, Glynn RJ, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA. 2009; 301:52–62. [PubMed: 19066368]
36. Stark JR, Perner S, Stampfer MJ, Sinnott JA, Finn S, Eisenstein AS, et al. Gleason score and lethal prostate cancer: does 3 + 4 = 4 + 3? J Clin Oncol. 2009; 27:3459–64. [PubMed: 19433685]
37. Park K, Tomlins SA, Mudaliar KM, Chiu YL, Esgueva R, Mehra R, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 2010; 12:590–8. [PubMed: 20651988]
38. Chaux A, Albadine R, Toubaji A, Hicks J, Meeker A, Platz E, A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol. 2011; 35:1014–20. [PubMed: 21677539]
39. van Leenders GJ, Boormans JL, Vissers CJ, Hoogland AM, Bressers AA, Furusato B, et al. Antibody EPR3864 is specific for ERG genomic fusions in prostate cancer: implications for pathological practice. Mod Pathol. 2011; 24:1128–38. [PubMed: 21499236]
40. Clark JP, Munson KW, Gu JW, Lamparska-Kupsik K, Chan KG, Yoshida JS, et al. Performance of a single assay for both type III and type VI TMPRSS2:ERG fusions in noninvasive prediction of prostate biopsy outcome. Clin Chem. 2008; 54:2007–17. [PubMed: 18948370]
41. Tomlins SA, Aubin SM, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. Sci Transl Med. 2011; 3:94ra72.

42. Zhang S, Pavlovitz B, Tull J, Wang Y, Deng FM, Fuller C. Detection of TMPRSS2 gene deletions and translocations in carcinoma, intraepithelial neoplasia, and normal epithelium of the prostate by direct fluorescence in situ hybridization. Diagn Mol Pathol. 2010; 19:151–6. [PubMed: 20736744]
43. Rajput AB, Miller MA, De Luca A, Boyd N, Leung S, Hurtado-Coll A, et al. Frequency of the TMPRSS2:ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol. 2007; 60:1238–43. [PubMed: 17259299]
44. Bismar TA, Dolph M, Teng LH, Liu S, Donnelly B. ERG protein expression reflects hormonal treatment response and is associated with Gleason score and prostate cancer specific mortality. Eur J Cancer. 2012; 48:538–46. [PubMed: 22300588]
45. Guo CC, Dancer JY, Wang Y, Aparicio A, Navone NM, Troncoso P, et al. TMPRSS2-ERG gene fusion in small cell carcinoma of the prostate. Hum Pathol. 2011; 42:11–7. [PubMed: 21040948]
46. Lotan TL, Toubaji A, Albadine R, Latour M, Herawi M, Meeker AK, et al. TMPRSS2-ERG gene fusions are infrequent in prostatic ductal adenocarcinomas. Mod Pathol. 2009; 22:359–65. [PubMed: 19151660]
47. Stott SL, Lee RJ, Nagrath S, Yu M, Miyamoto DT, Ulkus L, et al. Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. Sci Transl Med. 2010; 2:25ra3.
48. Han B, Suleman K, Wang L, Siddiqui J, Sercia L, Magi-Galluzzi C, et al. ETS gene aberrations in atypical cribriform lesions of the prostate: implications for the distinction between intraductal carcinoma of the prostate and cribriform high-grade prostatic intraepithelial neoplasia. Am J Surg Pathol. 2010; 34:478–85. [PubMed: 20220513]
49. Danila DC, Anand A, Sung CC, Heller G, Leversha MA, Cao L, et al. TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. Eur Urol. 2011; 60:897–904. [PubMed: 21802835]
50. Dhani NC, Emmenegger U, Adams L, Jongstra J, Tannock IF, Sridhar SS, et al. Phase II study of cytarabine in men with docetaxel-refractory, castration-resistant prostate cancer with evaluation of TMPRSS2-ERG and SPINK1 as serum biomarkers. BJU Int. 2012
51. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7:177–88. [PubMed: 3802833]
52. Paulo P, Barros-Silva JD, Ribeiro FR, Ramalho-Carvalho J, Jeronimo C, Henrique R, et al. FLI1 is a novel ETS transcription factor involved in gene fusions in prostate cancer. Genes Chromosomes Cancer. 2012; 51:240–9. [PubMed: 22081504]
53. Albadine R, Latour M, Toubaji A, Haffner M, Isaacs WB, Platz EA, et al. TMPRSS2-ERG gene fusion status in minute (minimal) prostatic adenocarcinoma. Mod Pathol. 2009; 22:1415–22. [PubMed: 19734849]
54. Bonaccorsi L, Nesi G, Nuti F, Paglierani M, Krausz C, Masieri L, et al. Persistence of expression of the TMPRSS2:ERG fusion gene after pre-surgery androgen ablation may be associated with early prostate specific antigen relapse of prostate cancer: preliminary results. J Endocrinol Invest. 2009; 32:590–6. [PubMed: 19494719]
55. Falzarano SM, Navas M, Simmerman K, Klein EA, Rubin MA, Zhou M, et al. ERG rearrangement is present in a subset of transition zone prostatic tumors. Mod Pathol. 2010; 23:1499–506. [PubMed: 20693982]
56. Falzarano SM, Zhou M, Hernandez AV, Klein EA, Rubin MA, Magi-Galluzzi C. Single focus prostate cancer: pathological features and ERG fusion status. J Urol. 2011; 185:489–94. [PubMed: 21167530]
57. FitzGerald LM, Agalliu I, Johnson K, Miller MA, Kwon EM, Hurtado-Coll A, et al. Association of TMPRSS2-ERG gene fusion with clinical characteristics and outcomes: results from a population-based study of prostate cancer. BMC Cancer. 2008; 8:230. [PubMed: 18694509]
58. Furusato B, Gao CL, Ravindranath L, Chen Y, Cullen J, McLeod DG, et al. Mapping of TMPRSS2-ERG fusions in the context of multi-focal prostate cancer. Mod Pathol. 2008; 21:67–75. [PubMed: 18065961]
59. Guo CC, Zuo G, Cao D, Troncoso P, Czerniak BA. Prostate cancer of transition zone origin lacks TMPRSS2-ERG gene fusion. Mod Pathol. 2009; 22:866–71. [PubMed: 19396154]

60. Laxman B, Tomlins SA, Mehra R, Morris DS, Wang L, Helgeson BE, et al. Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. Neoplasia. 2006; 8:885–8. [PubMed: 17059688]
61. Mehra R, Han B, Tomlins SA, Wang L, Menon A, Wasco MJ, et al. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: molecular evidence for an independent group of diseases. Cancer Res. 2007; 67:7991–5. [PubMed: 17804708]
62. Mehra R, Tomlins SA, Shen R, Nadeem O, Wang L, Wei JT, et al. Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer. Mod Pathol. 2007; 20:538–44. [PubMed: 17334343]
63. Barros-Silva JD, Ribeiro FR, Rodrigues A, Cruz R, Martins AT, Jeronimo C, et al. Relative 8q gain predicts disease-specific survival irrespective of the TMPRSS2-ERG fusion status in diagnostic biopsies of prostate cancer. Genes Chromosomes Cancer. 2011; 50:662–71. [PubMed: 21584900]
64. Boormans JL, Hermans KG, Made AC, van Leenders GJ, Wildhagen MF, Collette L, et al. Expression of the androgen-regulated fusion gene TMPRSS2-ERG does not predict response to endocrine treatment in hormone-naive, node-positive prostate cancer. Eur Urol. 2010; 57:830–5. [PubMed: 19716227]
65. Furusato B, Tan SH, Young D, Dobi A, Sun C, Mohamed AA, et al. ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification. Prostate Cancer Prostatic Dis. 2010; 13:228–37. [PubMed: 20585344]
66. Furusato B, van Leenders GJ, Trapman J, Kimura T, Egawa S, Takahashi H, et al. Immunohistochemical ETS-related gene detection in a Japanese prostate cancer cohort: diagnostic use in Japanese prostate cancer patients. Pathol Int. 2011; 61:409–14. [PubMed: 21707844]
67. Mani RS, Iyer MK, Cao Q, Brenner JC, Wang L, Ghosh A, et al. TMPRSS2-ERG-mediated feed-forward regulation of wild-type ERG in human prostate cancers. Cancer Res. 2011; 71:5387–92. [PubMed: 21676887]
68. Svensson MA, LaFargue CJ, MacDonald TY, Pflueger D, Kitabayashi N, Santa-Cruz AM, et al. Testing mutual exclusivity of ETS rearranged prostate cancer. Lab Invest. 2011; 91:404–12. [PubMed: 20975660]
69. Vainio P, Gupta S, Ketola K, Mirtti T, Mpindi JP, Kohonen P, et al. Arachidonic acid pathway members PLA2G7, HPGD, EPHX2, and CYP4F8 identified as putative novel therapeutic targets in prostate cancer. Am J Pathol. 2011; 178:525–36. [PubMed: 21281786]
70. Winnes M, Lissbrant E, Damber JE, Stenman G. Molecular genetic analyses of the TMPRSS2-ERG and TMPRSS2-ETV1 gene fusions in 50 cases of prostate cancer. Oncol Rep. 2007; 17:1033–6. [PubMed: 17390040]
71. Braun M, Scheble VJ, Menon R, Scharf G, Wilbertz T, Petersen K, et al. Relevance of cohort design for studying the frequency of the ERG rearrangement in prostate cancer. Histopathology. 2011; 58:1028–36. [PubMed: 21707704]
72. Hoogland AM, Jenster G, van Weerden WM, Trapman J, van der Kwast T, Roobol MJ, et al. ERG immunohistochemistry is not predictive for PSA recurrence, local recurrence or overall survival after radical prostatectomy for prostate cancer. Mod Pathol. 2012; 25:471–9. [PubMed: 22080055]
73. Esgueva R, Perner S, LaFargue C, Scheble V, Stephan C, Lein M, et al. Prevalence of TMPRSS2-ERG and SLC45A3-ERG gene fusions in a large prostatectomy cohort. Mod Pathol. 2010; 23:539–46. [PubMed: 20118910]
74. Greene DR, Fitzpatrick JM, Scardino PT. Anatomy of the prostate and distribution of early prostate cancer. Semin Surg Oncol. 1995; 11:9–22. [PubMed: 7754280]
75. Bismar TA, Trpkov K. TMPRSS2-ERG gene fusion in transition zone prostate cancer. Mod Pathol. 2010; 23:1040–1. author reply 1-2. [PubMed: 20596054]
76. Liu S, Yoshimoto M, Trpkov K, Duan Q, Firszt M, Corcos J, et al. Detection of ERG gene rearrangements and PTEN deletions in unsuspected prostate cancer of the transition zone. Cancer Biol Ther. 2011; 11:562–6. [PubMed: 21178509]
77. Sboner A, Demichelis F, Calza S, Pawitan Y, Setlur SR, Hoshida Y, et al. Molecular sampling of prostate cancer: a dilemma for predicting disease progression. BMC Med Genomics. 2010; 3:8. [PubMed: 20233430]

78. King CR, Ferrari M, Brooks JD. Prognostic significance of prostate cancer originating from the transition zone. Urol Oncol. 2009; 27:592–7. [PubMed: 18799332]

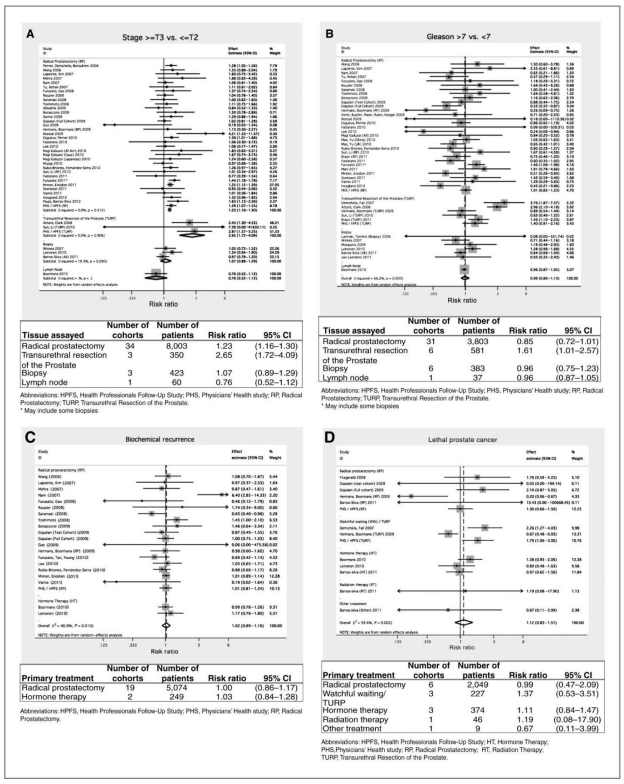
79. Shah RB, Chinnaiyan AM. The discovery of common recurrent transmembrane protease serine 2 (TMPRSS2)-erythroblastosis virus E26 transforming sequence (ETS) gene fusions in prostate cancer: significance and clinical implications. Adv Anat Pathol. 2009; 16:145–53. [PubMed: 19395877]

80. Brenner JC, Ateeq B, Li Y, Yocum AK, Cao Q, Asangani IA, et al. Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. Cancer Cell. 2011; 19:664–78. [PubMed: 21575865]

81. Swanson TA, Krueger SA, Galoforo S, Thibodeau BJ, Martinez AA, Wilson GD, et al. TMPRSS2/ERG fusion gene expression alters chemo- and radio-responsiveness in cell culture models of androgen independent prostate cancer. Prostate. 2011

82. Antonarakis ES, Chen Y, Elsamanoudi SI, Brassell SA, Da Rocha MV, Eisenberger MA, et al. Long-term overall survival and metastasis-free survival for men with prostate-specific antigen-recurrent prostate cancer after prostatectomy: analysis of the Center for Prostate Disease Research National Database. BJU Int. 2010

83. Freedland SJ, Humphreys EB, Mangold LA, Eisenberger M, Dorey FJ, Walsh PC, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. JAMA. 2005; 294:433–9. [PubMed: 16046649]



Tissue assayed	Number of cohorts	Number of patients	Risk ratio	95% CI
Radical prostatectomy	34	8,003	1.23	(1.16–1.30)
Transurethral resection of the Prostate	3	350	2.65	(1.72–4.09)
Biopsy	3	423	1.07	(0.89–1.29)
Lymph node	1	60	0.76	(0.52–1.12)

Abbreviations: HPFS, Health Professionals Follow-Up Study; PHS, Physicians' Health study; RP, Radical Prostatectomy; TURP, Transurethral Resection of the Prostate.
* May include some biopsies

Tissue assayed	Number of cohorts	Number of patients	Risk ratio	95% CI
Radical prostatectomy	31	3,803	0.85	(0.72–1.01)
Transurethral resection of the Prostate	6	581	1.61	(1.01–2.57)
Biopsy	6	383	0.96	(0.75–1.23)
Lymph node	1	37	0.96	(0.87–1.06)

Abbreviations: HPFS, Health Professionals Follow-Up Study; PHS, Physicians' Health study; RP, Radical Prostatectomy; TURP, Transurethral Resection of the Prostate.
* May include some biopsies

Primary treatment	Number of cohorts	Number of patients	Risk ratio	95% CI
Radical prostatectomy	19	5,074	1.00	(0.86–1.17)
Hormone therapy	2	249	1.03	(0.84–1.28)

Abbreviations: HPFS, Health Professionals Follow-Up Study; PHS, Physicians' Health study; RP, Radical Prostatectomy.

Primary treatment	Number of cohorts	Number of patients	Risk ratio	95% CI
Radical prostatectomy	6	2,049	0.99	(0.47–2.09)
Watchful waiting/ TURP	3	227	1.37	(0.53–3.51)
Hormone therapy	3	374	1.11	(0.84–1.47)
Radiation therapy	1	46	1.19	(0.08–17.90)
Other treatment	1	9	0.67	(0.11–3.99)

Abbreviations: HPFS, Health Professionals Follow-Up Study; HT, Hormone Therapy; PHS,Physicians' Health study; RP, Radical Prostatectomy; RT, Radiation Therapy; TURP, Transurethral Resection of the Prostate.

Figure 1.

Data on *TMPRSS2:ERG* fusion status as assessed by FISH, RT-PCR or IHC and risk of (A) advanced stage prostate cancer (>T3 vs. <T2), (B) high grade prostate cancer (Gleason 8-10 vs. Gleason 2-6), (C) biochemical recurrence, and (D) lethal prostate cancer (prostate cancer-specific death or development of distant metastases)

Table 1

Clinical Characteristics for all men and by ERG overexpression status among 1,180 men treated with radical prostatectomy for prostate cancer[a], PHS and HPFS cohorts 1983-2011

Characteristic	All Men	ERG Negative	ERG Positive	% ERG Positive	P[b]
Number	1,180	596	584	49%	
Mean Follow-Up Time (years ± SD)	12.6 ± 4.5	12.2 ±4.2	13.0 ±4.7	-	<0.01
Mean Age at Diagnosis (years ± SD)	65.4 ± 5.9	65.6 ±5.9	65.2 ±5.9	-	0.17
Pathological Tumor Stage					
T2 N0/Nx	818 (72%)	430 (75%)	388 (68%)	47%	
T3 N0/Nx	290 (25%)	130 (23%)	160 (28%)	55%	
T4/N1/M1	34 (3%)	12 (2%)	22 (4%)	65%	<0.01
Gleason Sum					
2−6	256 (22%)	135 (23%)	121 (21%)	47%	
3+4	438 (37%)	204 (34%)	234 (40%)	53%	
4+3	268 (23%)	143 (24%)	125 (21%)	47%	
8−10	218 (18%)	114 (19%)	104 (18%)	48%	0.58
PSA-Level at Diagnosis					
<4	132 (12%)	56 (10%)	76 (14%)	58%	
4-<10	641 (60%)	322 (60%)	319 (60%)	50%	
≥10	293 (27%)	160 (30%)	133 (25%)	45%	0.02
Lethal Prostate Cancer [c]					
No	1,095 (93%)	553 (93%)	542 (93%)	50%	
Yes	85 (7%)	43 (7%)	42 (7%)	49%	0.99
Biochemical Recurrence					
No	914 (77%)	462 (78%)	452 (77%)	49%	
Yes	266 (23%)	134 (22%)	132 (23%)	50%	0.96
All Cause Mortality [d]					
No	869 (74%)	435 (73%)	434 (74%)	50%	
Yes	311 (26%)	161 (27%)	150 (26%)	48%	0.60

Abbreviations: PSA – Prostate Specific Antigen; SD – Standard Deviation

[a] Numbers may not add up to 1,180 because men with missing information for a characteristic are not included in that characteristic

[b] P-values are based on t-test for follow-up time and age at diagnosis; Cochran-Armitage trend test for tumor stage, Gleason sum, and PSA-level at diagnosis; Chi-square test for lethal prostate cancer, biochemical recurrence, and all cause mortality

[c] Lethal prostate cancer includes metastases to distant organs or bone, and prostate cancer death

[d] All cause mortality includes prostate cancer death and death due to any other cause

Table 2

Hazard ratios (HRs) and 95% confidence intervals (CIs) for prostate cancer recurrence and death by ERG status among 1,180 men treated with radical prostatectomy for prostate cancer, PHS and HPFS cohorts 1983-2011

	Model 1[a]		Model 2[b]	
Characteristic	**HR**	**95% CI**	**HR**	**95% CI**
Lethal Prostate Cancer (No. Events: 85) [c]				
ERG Negative	1.00		1.00	
ERG Positive	0.93	(0.61, 1.43)	0.85	(0.55, 1.31)
Biochemical Recurrence (No. Events: 266)				
ERG Negative	1.00		1.00	
ERG Positive	0.99	(0.78, 1.26)	0.89	(0.70, 1.13)
All Cause Mortality (No. Events: 311) [d]				
ERG Negative	1.00		1.00	
ERG Positive	0.84	(0.67, 1.05)	0.84	(0.67, 1.05)

[a] Adjusted for age at diagnosis (<60, 60-64, 65-69, 70+), and cohort (PHS, HPFS)

[b] Adjusted for age at diagnosis (<60, 60-64, 65-69, 70+), cohort (PHS, HPFS), tumor stage (T2, T3, T4/N1/M1), and Gleason score (≤6, 3+4, 4+3, ≥8)

[c] Lethal prostate cancer includes metastases to distant organs or bone, and prostate cancer death

[d] All cause mortality includes prostate cancer death and death due to any other cause

Table 3

Prevalence of the *TMPRSS2:ERG* gene fusion in men with prostate cancer from studies in the meta-analysis

	Overall Fusion Analysis		Fusion Mechanism Analysis	
Characteristic	**Sample Size**	**Prevalence Fusion Positive**	**Sample Size**	**Prevalence Fusion-by-Deletion[a]**
Overall	10,779	46.9%	1,390	63.7%
Assay [b]				
FISH	3,146	42.4%	1,008	62.6%
RT-PCR	1,311	52.2%	N/A	N/A
IHC	4,763	51.8%	N/A	N/A
Cohort Continent [b c]				
Asia	837	23.4%	67	61.2%
Europe	4,926	53.6%	380	63.9%
North America	3,217	47.6%	530	63.2%
Tissue Assayed				
Biopsy	611	43.0%	190	60.0%
Lymph Node	71	59.2%	0	N/A
RP Specimen	9,220	48.6%	1,035	63.1%
TURP Specimen	803	29.8%	165	71.5%
Urine	74	36.5%	0	N/A

Abbreviations: FISH – Fluorescence In Situ Hybridization; IHC – Immunohistochemistry; RP – Radical Prostatectomy; RT-PCR – Reverse Transcription Polymerase Chain Reaction; TURP – Transurethral Resection of the Prostate

[a] Among those for whom fusion mechanism was determined

[b] In radical prostatectomy specimens only

[c] Excludes cohorts with patients from multiple regions or areas outside those listed