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 transmembrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (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 (hazard ratio [HR], 0.99; 95% confidence interval [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). TMPRSS2:ERG was associated with stage at diagnosis (risk ratio [RR], T3 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. Cancer Epidemiol Biomarkers Prev; 1–13. ©2012 AACR.
The gene fusion involves TMPRSS2 and ERG, 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), whereas 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, 12–23, 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 vs. watchful waiting cohorts), tumor tissue assessed for heterogeneity of study cohorts (e.g., radical prostatectomy number of events in most prior studies, as well as by explained in part by the small sample sizes and limited

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 after surgery sustained over 2 measures. The date of first increase 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. Lis, R. Flavin, M. Fiorcntino, and M. Loda) reviewed hematoxylin and cosin slides to provide uniform Gleason grade and other histopathologic 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 12 tissue microarrays.

Materials and Methods

Cohort study

Study population. The study was nested among U.S. 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 to 75 years at enrollment in 1986. Men in both studies were free of diagnosed cancer, excluding nonmelanoma skin cancer, at baseline.

The aims 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.

Cancer Epidemiology Biomarkers & Prevention
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 4x00 automated staining platform (BioGenex Laboratories Inc.). Five-micrometer formalin-fixed, paraffin-embedded sections of each tissue microarray were deparaffinized in xylene, followed by a graded alcohol dehydration. Antigen retrieval was conducted by microwaving the tissue in citrate buffer for 5 minutes. ERG antisera (Clone ID: EPR3864, Epitomics, Inc.) were 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.). 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. Lis). 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 restained 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. 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 Supplementary Materials (Supplementary Table S1).

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 χ² tests or Cochran–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 hazard models to calculate hazard ratios (HRs) and 95% confidence intervals (CI). We defined prostate cancer progression in two ways: (i) time to lethal prostate cancer, defined as development of distant metastases or prostate cancer death, and (ii) time to biochemical recurrence. Men who did not report a PSA increase 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 1 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.). All tests were 2 sided with P < 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 (pathologic 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 article (1)], and we conducted 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 articles 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 articles discovered during the June 2010 search, as well as Minner and colleagues (because of the study’s large sample size; ref. 26), and received additional data for 16.

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; refs. 40, 41). We also omitted studies in which no outcomes of interest occurred (n = 1; ref. 42). Studies containing inconsistencies in the data presented in the article were excluded as well (n = 2; refs. 43, 44), as were cohorts looking at certain subsets of prostate cancer (n = 5; refs. 45–49). We excluded one further study for which we could not determine the procedure used to collect tumor tissue (n = 1; ref. 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. Pettersson and R.E. Graff) 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 (RR) as the measure of association between fusion status and tumor stage (T3 or greater compared with T2 or less), Gleason score (Gleason 8–10 compared with Gleason 2–6 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 (WMD) as the measure of association with fusion status. Age analyses were restricted to the subset of studies for which standard deviation (SD) of the means in each exposure group were available (Ten cohorts had mean ages in each exposure group but did not have SD 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, and 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; ref. 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). All tests were 2 sided with P < 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, 47–86). 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 < 0.01); the prevalence of tumors overexpressing ERG was 65% among men with pT4/N1/M1 tumors compared with 47% among men with pT2 tumors. We found no association between ERG...
overexpression and Gleason score ($P = 0.58$), or with age at diagnosis ($P = 0.17$). However, ERG overexpression was associated with lower PSA level at diagnosis ($P = 0.02$), and associated with a slightly longer mean follow-up time (13.0 vs. 12.2 years; $P < 0.01$). 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 after 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**

Supplementary Figure S1 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 Supplementary Table S2. Studies that were reviewed, but excluded, are described in Supplementary Table S3. 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All men</th>
<th>ERG-negative</th>
<th>ERG positive</th>
<th>% ERG positive</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1,180</td>
<td>596</td>
<td>584</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Mean follow-up time (years ± SD)</td>
<td>12.6 ± 4.5</td>
<td>12.2 ± 4.2</td>
<td>13.0 ± 4.7</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean age at diagnosis (years ± SD)</td>
<td>65.4 ± 5.9</td>
<td>65.6 ± 5.9</td>
<td>65.2 ± 5.9</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Pathological tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 N0/Nx</td>
<td>818 (72%)</td>
<td>430 (75%)</td>
<td>388 (68%)</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>T3 N0/Nx</td>
<td>290 (25%)</td>
<td>130 (23%)</td>
<td>160 (28%)</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>T4/N1/M1</td>
<td>34 (3%)</td>
<td>12 (2%)</td>
<td>22 (4%)</td>
<td>65%</td>
<td>&lt;0.01</td>
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<tr>
<td>Gleason sum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>256 (22%)</td>
<td>135 (23%)</td>
<td>121 (21%)</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>3–4</td>
<td>438 (37%)</td>
<td>204 (34%)</td>
<td>234 (40%)</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>4–3</td>
<td>268 (23%)</td>
<td>143 (24%)</td>
<td>125 (21%)</td>
<td>47%</td>
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<td>8–10</td>
<td>218 (18%)</td>
<td>114 (19%)</td>
<td>104 (18%)</td>
<td>48%</td>
<td>0.58</td>
</tr>
<tr>
<td>PSA level at diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>132 (12%)</td>
<td>56 (10%)</td>
<td>76 (14%)</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>4–&lt;10</td>
<td>641 (60%)</td>
<td>322 (60%)</td>
<td>319 (60%)</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>293 (27%)</td>
<td>160 (30%)</td>
<td>133 (25%)</td>
<td>45%</td>
<td>0.02</td>
</tr>
<tr>
<td>Lethal prostate cancer$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,095 (93%)</td>
<td>553 (93%)</td>
<td>542 (93%)</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>85 (7%)</td>
<td>43 (7%)</td>
<td>42 (7%)</td>
<td>49%</td>
<td>0.99</td>
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<tr>
<td>Biochemical recurrence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>914 (77%)</td>
<td>462 (78%)</td>
<td>452 (77%)</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>266 (23%)</td>
<td>134 (22%)</td>
<td>132 (23%)</td>
<td>50%</td>
<td>0.96</td>
</tr>
<tr>
<td>All-cause mortality$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>869 (74%)</td>
<td>435 (73%)</td>
<td>434 (74%)</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>311 (26%)</td>
<td>161 (27%)</td>
<td>150 (26%)</td>
<td>48%</td>
<td>0.60</td>
</tr>
</tbody>
</table>

**NOTE:** Numbers may not add up to 1,180 because men with missing information for a characteristic are not included in that characteristic.

$^a$ $P$ 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; $^b$ $x^2$ test for lethal prostate cancer, biochemical recurrence, and all-cause mortality.

$^b$ Lethal prostate cancer includes metastases to distant organs or bone, and prostate cancer death.

$^c$ All-cause mortality includes prostate cancer death and death due to any other cause.
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 patients with prostate cancer 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).

Figure 1A–D presents 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 vs. T2 or lower; Fig. 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). Fig. 1C and D presents 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 $I^2$ 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 ($P = 0.04$), 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 RR comparing Gleason 8 to 10 versus 2 to 6 tumors was 0.83 (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 (Fig. 1C and D). Results for age were also consistent with the overall analysis (WMD: −0.66 years; 95% CI, −1.34 to 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 2 times as likely to be diagnosed with cancers at a higher clinical stage (RR, 2.65; 95% CI, 2.00–3.54).

### Table 2. HRs and 95% CIs for prostate cancer recurrence and death by ERG status among 1,180 men treated with radical prostatectomy for prostate cancer, Physicians’ Health Study, and Health Professionals Follow-Up Study cohorts 1983–2011

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Model 1a (HR (95% CI))</th>
<th>Model 2b (HR (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal prostate cancer (no. events: 85)d</td>
<td>1.00 (1.00)</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>ERG negative</td>
<td>1.00 (0.61–1.43)</td>
<td>0.85 (0.55–1.31)</td>
</tr>
<tr>
<td>ERG positive</td>
<td>0.93 (0.61–1.43)</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>Biochemical recurrence (no. events: 266)</td>
<td>1.00 (1.00)</td>
<td>0.89 (0.70–1.13)</td>
</tr>
<tr>
<td>ERG negative</td>
<td>0.99 (0.78–1.26)</td>
<td></td>
</tr>
<tr>
<td>ERG positive</td>
<td>1.00 (0.84–1.26)</td>
<td>0.84 (0.67–1.05)</td>
</tr>
<tr>
<td>All-cause mortality (no. events: 311)d</td>
<td>1.00 (1.00)</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>ERG negative</td>
<td>0.84 (0.67–1.05)</td>
<td>0.84 (0.67–1.05)</td>
</tr>
<tr>
<td>ERG positive</td>
<td>1.00 (1.00)</td>
<td>1.00 (1.00)</td>
</tr>
</tbody>
</table>

aAdjusted for age at diagnosis (<60, 60–64, 65–69, 70+), and cohort (Physicians’ Health Study, Health Professionals Follow-Up Study).

bAdjusted for age at diagnosis (<60, 60–64, 65–69, 70+), cohort (Physicians’ Health Study, Health Professionals Follow-Up Study), tumor stage (T2, T3, T4/N1/M1), and Gleason score (<6, 6–3, 4–4, 4–3, ≥8).

cLethal prostate cancer includes metastases to distant organs or bone, and prostate cancer death.

dAll-cause mortality includes prostate cancer death and death due to any other cause.
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). Although 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 (Fig. 1D). This finding is consistent with our cohort of 90 men diagnosed by TURP (Supplementary Table S1). Results for mean age at diagnosis were null (WMD: 0.16, 95% CI, 0.12 to 0.79).

Data on outcomes broken out by mechanism of the fusion (e.g., positive by deletion vs. 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 (Supplementary Table S4).

Among men treated with radical prostatectomy, 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 ($P = 0.06$).

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

### Table 3. Prevalence of the TMPRSS2:ERG gene fusion in men with prostate cancer from studies in the meta-analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sample size</th>
<th>Prevalence fusion positive</th>
<th>Sample size</th>
<th>Prevalence fusion-by-deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall fusion analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>10,779</td>
<td>46.9%</td>
<td>1,390</td>
<td>63.7%</td>
</tr>
<tr>
<td>Assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>3,146</td>
<td>42.4%</td>
<td>1,008</td>
<td>62.6%</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>1,311</td>
<td>52.2%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IHC</td>
<td>4,763</td>
<td>51.8%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cohort continent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>837</td>
<td>23.4%</td>
<td>67</td>
<td>61.2%</td>
</tr>
<tr>
<td>Europe</td>
<td>4,926</td>
<td>53.6%</td>
<td>380</td>
<td>63.9%</td>
</tr>
<tr>
<td>North America</td>
<td>3,217</td>
<td>47.6%</td>
<td>530</td>
<td>63.2%</td>
</tr>
<tr>
<td>Tissue assayed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>611</td>
<td>43.0%</td>
<td>190</td>
<td>60.0%</td>
</tr>
<tr>
<td>Lymph node</td>
<td>71</td>
<td>59.2%</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>RP specimen</td>
<td>9,220</td>
<td>48.6%</td>
<td>1,035</td>
<td>63.1%</td>
</tr>
<tr>
<td>TURP specimen</td>
<td>803</td>
<td>29.8%</td>
<td>165</td>
<td>71.5%</td>
</tr>
<tr>
<td>Urine</td>
<td>74</td>
<td>36.5%</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: RP, radical prostatectomy.

*Among those for whom fusion mechanism was determined.

In radical prostatectomy specimens only.

Excludes cohorts with patients from multiple regions or areas outside those listed.
<table>
<thead>
<tr>
<th>Tissue assayed</th>
<th>Number of cohorts</th>
<th>Number of patients</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radical prostatectomy</td>
<td>34</td>
<td>8,003</td>
<td>1.23 (1.16–1.30)</td>
</tr>
<tr>
<td>Transurethral resection of the Prostate</td>
<td>3</td>
<td>350</td>
<td>2.65 (1.72–4.09)</td>
</tr>
<tr>
<td>Biopsy</td>
<td>3</td>
<td>423</td>
<td>1.07 (0.89–1.29)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>1</td>
<td>60</td>
<td>0.76 (0.52–1.12)</td>
</tr>
</tbody>
</table>

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

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 >7 vs. ≤7).
Figure 1. (Continued) (C) biochemical recurrence, and (D) lethal prostate cancer (prostate cancer-specific death or development of distant metastases).
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; refs. 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 and colleagues found an almost 3-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 with men with fusion-negative prostate cancer (10). Only 3 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 with 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.

It has been suggested that cancers harboring gene fusions occurring by deletion have worse prognosis than those occurring by translocation, possibly because the approximately 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 downregulation of tumor suppressor genes as well as upregulation 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 vs. 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, including 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 CI of the pooled RR. 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 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 cancerspecific 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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Accumulation of data (provided animals, acquired and managed patients, provided facilities, etc.): R.T. Lis, E.C. Stack, A.H. Ligon, C. Suppan, R. Flavin, H.D. Sesso, M.J. StAMPLer, E.L. Giovannucci, M. Loda, L.A. Mucci


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.T. Lis, H.D. Sesso, J.R. Rider, M.J. StAMPLer

Study supervision: M.J. StAMPLer, M.G. Sanda, M. Loda, L.A. Mucci

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The TMPRSS2:ERG Rearrangement, ERG Expression, and Prostate Cancer Outcomes: A Cohort Study and Meta-analysis

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