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Prevalence of *TMPRSS2-ERG* fusion prostate cancer among men undergoing prostate biopsy in the United States

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Abstract

Purpose—Fusion of the *TMPRSS2* prostate-specific gene with the *ERG* transcription factor is a putatively oncogenic gene rearrangement that is commonly found in prostate cancer tissue from men undergoing prostatectomy. However, the prevalence of the fusion was less common in TURP samples from a Swedish cohort of incidental prostate cancer patients followed by watchful waiting, raising the question as to whether the high prevalence in prostatectomy specimens reflects selection bias. We sought to determine the prevalence of *TMPRSS2-ERG* gene fusion among PSA-screened men undergoing prostate biopsy in the United States.

Experimental design—We studied 140 prostate biopsies from the same number of patients for *TMPRSS2-ERG* fusion status with a FISH assay. 134 (100 cancer and 34 benign) were assessable.

Results—*ERG* gene rearrangement was detected in 46% prostate biopsies that were found to have prostate cancer and in 0% of benign prostate biopsies ($p < 0.0001$). Evaluation of morphological features showed that cribriform growth, blue-tinged mucin, macronucleoli and

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collagenous micronodules were significantly more frequent in *TMPRSS2-ERG* fusion positive prostate cancer biopsies than gene fusion negative prostate cancer biopsies ($p = 0.04$). No significant association with Gleason score was detected. In addition, non-Caucasian patients were less likely to have positive fusion status ($p=0.02$).

Conclusions—This is the first prospective North American multi-center study to characterize the *TMPRSS2-ERG* prostate cancer prevalence in a cohort of patients undergoing needle biopsy irrespective of whether or not they subsequently undergo prostatectomy. Our results show that this gene rearrangement is common among North American men who have prostate cancer on biopsy, is absent in benign prostate biopsy, and is associated with specific morphological features. These findings indicate a need for prospective studies to evaluate the relationship of *TMPRSS2-ERG* rearrangement with clinical course of screening-detected prostate cancer in North American men, and development of non-invasive screening tests to detect *TMPRSS2-ERG* rearrangement.

Introduction

Since its initial discovery (1), the prevalence of *TMPRSS2-ERG* prostate cancer was reported to vary. In a German cohort where prostatectomy specimens were studied, this approached 50% whereas in an incidental watchful-waiting cohort from Sweden, the prevalence was close to 15% in transurethral resection of prostate (TURP) samples (2, 3). A subsequent study from Portugal confirmed 50% of *TMPRSS2-ERG* prostate cancer in patients who underwent radical prostatectomy for clinically localized disease (4). In more recent publications from Canada and the United States, the prevalence in prostatectomies ranges between 36% (including all Gleason scores) and 54%, respectively (5, 6). A question has been raised as to whether the high prevalence in prostatectomy specimens is due to selection bias or genetic differences between populations. Further, systematic studies documenting the prevalence of *TMPRSS2-ERG* gene fusion in North American cohorts undergoing prostate biopsy are lacking.

In the United States, approximately 1,300,000 prostate biopsies are performed each year, and only in 2006, 234,460 new cases of prostate cancer were diagnosed (American Cancer Society, Cancer facts & figures 2006). The American Cancer Society estimates that 186,320 men will be diagnosed with prostate cancer in 2008 and about 28,660 will die from it, making it the most common noncutaneous cancer and the second most lethal cancer among men in the United States (American Cancer Society, Cancer facts & figures 2008). Emerging data suggests that *TMPRSS2-ERG* prostate cancer has a worse prognosis. Specifically, higher tumor stage and tumor-specific death or metastases have been documented (2, 3, 6–8).

The purpose of this study was to assess the *TMPRSS2-ERG* gene fusion status among PSA-screened men undergoing prostate biopsy in the United States. Given the most recent advances in the evolving story of *TMPRSS2-ERG* prostate cancer, our work may impact the clinical management of patients with fusion positive prostate cancer detected on needle biopsies.

Materials and Methods

Cohort selection

The analysis involved 140 consecutive patients enrolled in the IRB-approved Early Detection Research Network (EDRN) study from five separate urological practices in Massachusetts and Michigan. Eligible patients were men referred for consideration of prostate biopsy due to either abnormal rectal exam or elevated PSA, or clinical suspicion of prostate cancer. The biopsy paraffin blocks were available for analysis and all corresponding

H&E stained slides (three slides per paraffin block) were reviewed. A representative slide from each patient was selected for evaluation of *TMPRSS2-ERG* gene fusion by fluorescent *in situ* hybridization (FISH) (see below). Of the 140 total evaluated biopsy subjects, available histology tissue from 6 was non-assessable by FISH. Of the remaining 134 subjects' biopsy samples, 100 had prostate cancer and 34 were exclusively benign. The latter included 25 benign biopsies from subjects without prostate cancer and 9 benign biopsies from subjects with prostate cancer. Table 1 summarizes the clinical characteristics of the cohort included in the study.

Pathologic analysis

The morphological diagnosis was confirmed on H&E slides (twelve biopsies per patient) by five pathologists (JMM, EMG, SP, RM, and RBS) prior to evaluation of the *TMPRSS2-ERG* fusion status. All positive cases were acinar prostate cancer. The Gleason score for each case was assessed and the morphological features below were evaluated blinded to the *TMPRSS2-ERG* fusion status. Evaluation of morphological features was performed by two independent groups composed of two observers each (JMM and SP, and RM and RBS). Common morphological features of prostate cancer were assessed as previously described (9). These included intraluminal features (blue-tinged mucin), nuclear features (macronucleoli), architectural features (intraductal tumor spread, cribriform growth pattern), malignant-specific features (extraprostatic extension, perineural invasion, glomerulations, and collagenous micronodules), histological variants (signet-ring cell features, foamy gland morphology), and comedonecrosis.

Determination of *TMPRSS2-ERG* fusion status

We have previously described a dual-color interphase break-apart FISH assay to detect the fusion of *TMRSS2-ERG* (1, 3, 10). Briefly, the two probes used are differentially labeled and span the telomeric and centromeric neighboring regions of the *ERG* locus. Because the two genes are close together on chromosome 21, a break-apart probe system has demonstrated to be accurate. The centromeric probe RP11-24A11 overlaps the 3' *ERG* coding region, and the telomeric probe RP11137J13 localizes to the intervening region between *ERG* and *TMPRSS2*. With this system, a nucleus without *ERG* rearrangement demonstrates two pairs of juxtaposed red and green signals, forming yellow signals. A nucleus with *TMPRSS2-ERG* fusion through insertion shows split-apart of one juxtaposed red-green signal pair resulting in single red and green signals for the translocated *ERG* allele, and a still combined (yellow) signal for the non-rearranged *ERG* allele in each nucleus. If *TMPRSS2-ERG* fusion occurs through interstitial deletion of genetic material, only two signals are detected in each nucleus: a yellow signal (for the non-rearranged), and a single red signal for the rearranged allele.

The samples were analyzed under a 60× oil immersion objective using an Olympus BX-51 fluorescence microscope equipped with appropriate filters, a CCD (charge-coupled device) camera (Olympus, Center Valley, PA), and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA). Evaluation of the tests was independently performed by four pathologists, (JMM, SP, RM and RBS). At one institution two pathologists (JMM and SP) evaluated 99 biopsies, and at the other institution two pathologists (RM and RBS) evaluated 41 biopsies. For each biopsy, we attempted to score at least 50 nuclei.

To ascertain consistency between institutions in evaluation of pathology and *TMPRSS2-ERG* fusion status

A subset of 30 biopsies (16 from Institution 1 and 14 from Institution 2) was exchanged for validation of the results. For each case, one H&E slide and the corresponding FISH slide

were sent for evaluation of morphological features, Gleason score and *TMPRSS2-ERG* fusion status. 26 FISH slides were assessable. Of the 26 pairs of assessable slides exchanged for cross evaluation among pathologists at the two institutions, there was complete agreement on the *TMPRSS2-ERG* fusion status by FISH in all cases. The fluorescent signals or the remaining 4 cases were faded. Signal enhancing was attempted but excessive background limited the interpretation. After re-cutting these 4 cases to repeat FISH, the initial small foci of cancer were not present for evaluation.

Statistical analysis

Exact binomial 95% confidence intervals (CI) were calculated for prevalence among cancer and benign biopsies and compared using Fisher's exact test. Among cancer biopsies, associations of clinical, histological and morphological features with *TMPRSS2-ERG* were assessed with Fisher's exact and Wilcoxon rank sum tests. A multivariable logistic regression analysis investigated which factors were most strongly associated with *TMPRSS2-ERG* fusion positive status.

Results

From among the 100 prostate cancer biopsies evaluable by FISH, 46 (46%; 95% CI 36%–56%) demonstrated *TMPRSS2-ERG* gene fusion. In the fusion positive prostate cancers, 63% of cases showed fusion through deletion and 37% showed fusion through insertion. All 34 benign biopsies, which included 9 benign biopsies from patients with prostate cancer, were fusion negative (95% CI 0–10%; $P < 0.001$ vs. cancer biopsies). In addition, normal prostatic glands adjacent to cancer areas in the same biopsy were negative for *TMPRSS2-ERG* gene fusion (Table 2). All histological features were evaluated for their association with *TMPRSS2-ERG* fusion status. After univariate statistical analysis, the following morphological features were determined to be associated with positive *TMPRSS2-ERG* fusion status: Cribriform growth ($p = 0.03$), blue-tinged mucin ($p < 0.01$), macronucleoli ($p = 0.02$), and collagenous micronodules ($p = 0.04$). Table 3 summarizes the findings of evaluation of morphological features in all 100 cancer-positive cases, and Figure 1 illustrates those with significant association with *TMPRSS2-ERG* fusion positive status. The presence of one or more of the above-mentioned morphological features was also associated with a positive *TMPRSS2-ERG* status, as fusion positive cases had more morphological features noted ($P < 0.01$ for the sum). No significant association was found between Gleason score and *TMPRSS2-ERG* fusion status (Table 4). When clinical characteristics were analyzed, non-Caucasian patients were less likely to have *TMPRSS2-ERG* fusion positive prostate cancer (13% vs. 52% fusion positive, $P = 0.02$) (Supplemental Table 1).

Multivariable analysis demonstrated that lower PSA density, cribriform growth pattern, blue-tinged mucin and macronucleoli were most strongly associated with *TMPRSS2-ERG* fusion status (Table 5). None of the aforementioned significant associations with positive *TMPRSS2-ERG* fusion status correlated with the mechanism of gene rearrangement, either through deletion or through insertion. Although not systematically sought, FISH identified four cases of *TMPRSS2-ERG* gene fusion HGPIN that shared the same fusion pattern with the prostate cancer in the same biopsy, and three cases of *TMPRSS2-ERG* gene fusion heterogeneity in prostate cancer, corresponding to separate areas of tumor within the same tissue core.

Discussion

The high prevalence of *TMPRSS2-ERG* prostate cancer is clinically relevant since emerging data suggests a worse prognosis of tumors harboring the gene fusion. Several studies have demonstrated that *TMPRSS2-ERG* fusion prostate cancer is associated with higher tumor

stage and tumor-specific death or metastasis (2, 3, 5, 6, 8, 11). One of the most relevant studies included men diagnosed with clinically localized prostate cancer in the pre-PSA era and followed with expectant management. In that study we observed that the presence of the *TMPRSS2-ERG* gene fusion was associated with either the development of prostate cancer metastases or prostate cancer specific death after up to 22 years of clinical follow up (2). More recently, Attard *et al* have described extremely poor cause-specific survival in patients whose prostate cancers demonstrate duplication of *TMPRSS2-ERG* fusion and interstitial deletion of sequences 5' to ERG, during an 8-year follow-up (7).

In the current study of a PSA-screened U.S. cohort, we demonstrate that *TMPRSS2-ERG* fusion prostate cancer has a prevalence of 46% in prostate needle biopsies, similar to that observed in radical prostatectomy specimens (3–6). Therefore, the higher prevalence of *TMPRSS2-ERG* prostate cancer observed in prostatectomy specimens from Portugal, Germany, and the United States (3–6) compared to transurethral prostate tissue from Sweden and United Kingdom (2, 7) is probably due to the high frequency of clinically insignificant tumors in the latter, and not due to genetic differences in the populations. In fact, 71 prostate biopsies with cancer from Sweden assessed by FISH demonstrated a 45% prevalence of *TMPRSS2-ERG* prostate cancer with the same distribution of mechanism of gene rearrangement seen in our study, that is 62.5% of cases through deletion and 37.5% of cases through insertion (12).

We have previously described five morphological features associated with *TMPRSS2-ERG* fusion prostate cancer (9). In the current study, cribriform growth pattern, blue-tinged mucin and macronucleoli were confirmed to be associated with a positive fusion status. The presence of collagenous micronodules that previously did not result as independently significant ($p = 0.056$) (9) has now been associated with *TMPRSS2-ERG* fusion prostate cancer. In the most recent USCAP meetings there have been controversial findings regarding the morphologic correlates of *TMPRSS2-ERG* fusion prostate cancer. Fine *et al* did not find any correlation (13) and Nigwekar *et al* found a significant association between perineural invasion, blue-tinged mucin and intraductal tumor spread with a positive gene fusion status (14).

The cross evaluation of the FISH assay by two independent groups of pathologists showed complete agreement. Minor discrepancies in Gleason grading and presence or absence of morphological features were observed. However, only one H&E slide of the 30 cases was available. This certainly limited the complete evaluation of each case, which included up to 12 core biopsies. Hence, FISH and morphological evaluation of *TMPRSS2-ERG* fusion prostate cancer could potentially be standardized. A future study will focus on the validation of these findings.

Although out of the scope of this manuscript, we identified four cases of *TMPRSS2-ERG* gene fusion HGPIN that shared the same fusion pattern with the prostate cancer in the same biopsy, and three cases of *TMPRSS2-ERG* gene fusion heterogeneity, corresponding to separate areas of prostate cancer within the same tissue core (data not shown). These observations are consistent with the most recent work on *TMPRSS2-ERG* fusion HGPIN and *TMPRSS2-ERG* fusion prostate cancer heterogeneity by our group and others (5, 15, 16). *TMPRSS2-ERG* interfocal clonal heterogeneity occurs in 41% of prostate cancers that are at least pT2c (15). Hence, FISH analysis would be necessary in bilateral prostate cancer positive cores if one result was negative. We have also demonstrated that *TMPRSS2-ERG* gene rearrangement, observed in approximately 20% of HGPIN lesions (4, 10, 16), is always indicative of *TMPRSS2-ERG* fusion prostate cancer (16).

Given their significant clinical implication, these are findings that merit consideration when *TMPRSS2-ERG* fusion status assessment on prostate biopsies is implemented, and in view of the development of a urine-based screening test for fusion transcripts (17–19).

Clinically, two factors may play a significant role in predicting the *TMPRSS2-ERG* fusion status in prostate cancer, race and PSA density. Non-Caucasian patients were less likely to have positive fusion status, and this association was significant ($p=0.02$). Preliminary data from a collaborative study with our group (20) including a larger number of Non-Caucasians show that *TMPRSS2-ERG* fusion prostate cancer is more common in Caucasians compared to African American and Asian patients ($p=0.034$). Further, the mechanism of *TMPRSS2-ERG* fusion through deletion, which has been associated with worse prognosis (2), is more common in prostate cancer of African American patients ($p=0.098$).

Interestingly, we have seen that one of the best predictors for a positive *TMPRSS2-ERG* fusion status is a lower PSA density (Table 5).

In summary, we have assessed the *TMPRSS2-ERG* fusion status in a large series of prostate needle core biopsies in the United States. We have confirmed prevalence similar to previously reported series in prostatectomy specimens and also confirmed morphological features of *TMPRSS2-ERG* fusion positive prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Statement of Translational Relevance

The high prevalence of *TMPRSS2-ERG* prostate cancer is clinically relevant since emerging data suggests a worse prognosis of tumors harboring the gene fusion (i.e. higher tumor stage and tumor-specific death or metastasis). Prevalence of the *TMPRSS2-ERG* gene rearrangement in the U.S. has previously been described only in single-institution studies of archival, retrospective tissue banks comprised of prostate cancer tissue from patients who underwent prostatectomy (and less than 35% of prostate cancer diagnosed in the U.S. is treated by prostatectomy). Thus, the actual prevalence of this fusion event among the entire spectrum of biopsy-confirmed prostate cancers in the U.S. population is lacking, and no American multi-center data regarding this gene alteration has been described. In the current study of a PSA-screened U.S. cohort, we demonstrate that *TMPRSS2-ERG* fusion prostate cancer has a prevalence of 46% in prostate needle biopsies and show morphological features associated with the gene fusion. Our findings: 1) validate the feasibility of measuring *TMPRSS2-ERG* fusion in routine, clinical prostate biopsy samples; 2) demonstrate histo-morphological consequences of the gene rearrangement, and 3) provide a benchmark of the *TMPRSS2-ERG* rearrangement prevalence in a multi-center, U.S. cohort that reflects the spectrum of prostate cancer diagnosed in the U.S.

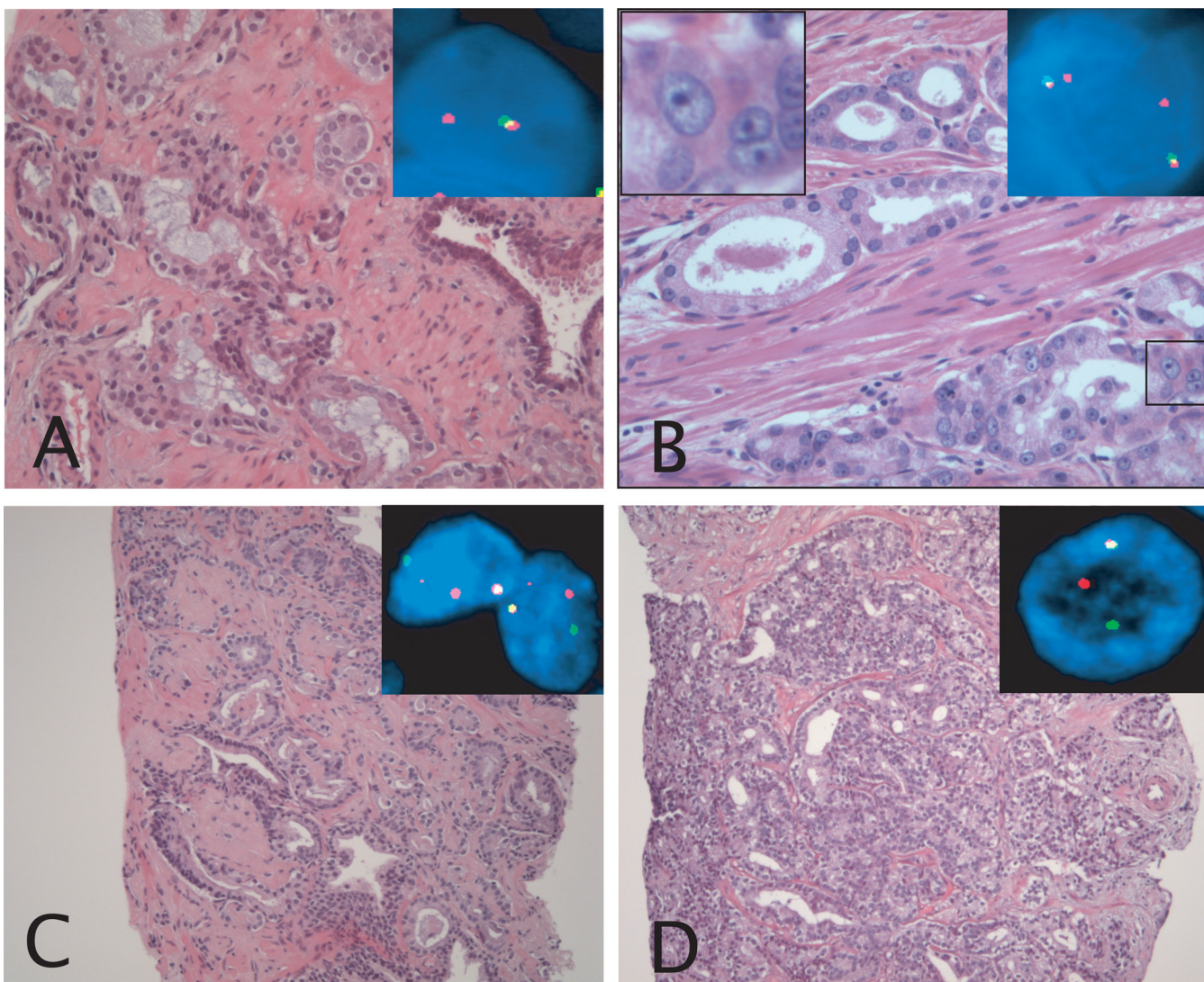


Figure 1. Morphological features associated with a positive *TMPRSS2-ERG* fusion status in prostate cancer. **A:** Prostate cancer Gleason pattern 3 showing blue-tinged mucin. Inset picture shows FISH image of representative nucleus. One yellow and one red signal are present, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. **B:** Prostate cancer Gleason pattern 3 showing macronucleoli. The inset picture in the upper left corner shows the macronucleoli in the boxed area, and the inset picture in the upper right corner shows a FISH image of representative nuclei. One yellow and one red signal are present in each nucleus, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. **C:** Prostate cancer Gleason pattern 4 with collagenous micronodules. Inset pictures shows FISH image of representative nuclei. One yellow, and separate red and green signals are present in each nucleus, demonstrating the presence of *TMPRSS2-ERG* fusion through insertion. **D:** Prostate cancer Gleason pattern 4 with cribriform growth pattern. Inset picture shows FISH image of representative nucleus. One yellow and separate red and green signals are present, demonstrating the presence of *TMPRSS2-ERG* fusion through insertion. Original magnification of H&E images, 20× objective (A and B), and 10× objective (C and D). Original magnification of FISH images, 60× objective.

Table 1

Clinical variables of the cohort of 134 men undergoing prostate needle biopsy at two institutions in the United States.

Variable	Institution*		All
	Institution 1 BIDMC	Institution 2 UM	
All patients	94 (70%)	40 (30%)	134 (100%)
Age (years)	65 [60–70]	61 [54–70]	64 [58–70]
Race[†]			
Caucasian	78 (83%)	35 (88%)	113 (84%)
Non-Caucasian	16 (17%)	5 (12%)	21 (16%)
PSA (ng/mL)	5.0 [4.0–7.0]	5.8 [4.7–10.0]	5.2 [4.0–8.0]
Prostate size by TRUS (cc)	43 [31–57]	43 [30–56]	43 [30–57]
PSA density (ng/mL/cc)	0.12 [0.08–0.19]	0.13 [0.09–0.24]	0.12 [0.08–0.19]
Number of cores taken	12 [12–12]	12 [12–12]	12 [12–12]
Number of cores involved	2 [1–4]	10 [1–12]	2 [1–6]
Percent of cores involved	17 [8–33]	77 [8–100]	17 [8–50]
Prostate cancer diagnosis			
No	17 (18)	8 (20)	25 (19)
Yes	77 (82)	32 (80)	109 (81)
Laterality			
Bilateral	23 (24)	23 (58)	46 (34)
Left	25 (27)	2 (5)	27 (20)
Right	28 (30)	1 (3)	29 (22)
Unknown	1 (1)	6 (16)	7 (5)
No cancer diagnosis	17 (18)	8 (20)	25 (19)
Gleason score			
Gleason 6	27 (29)	13 (33)	40 (30)
Gleason 7	39 (41)	11 (28)	50 (37)
Gleason 8	8 (8)	7 (18)	15 (11)
Not applicable or Unknown	20 (21)	9 (23)	29 (22)

* BIDMC = Beth Israel Deaconess Medical Center; UM = University of Michigan

Abbreviations: PSA = Prostate specific antigen; TRUS = transrectal ultrasound

[†] See Supplemental Table 1 for results on ethnicity by *TMPRSS2-ERG* fusion status among 100 patients' prostate cancer-positive biopsy cores

Table 2
TMPRSS2-ERG fusion status of 100 patients' cancer-positive biopsy cores at the two participating institutions.

	Institution *						Total	
	Institution 1 BIDMC		Institution 2 UM					
	N	%	N	%	N	%	N	%
All cores	71	100	29	100	100	100	100	100
<i>TMPRSS2-ERG</i>								
Negative	41	58	13	45	54	54	54	54
Positive	30	42	16	55	46	46	46	46
All <i>TMPRSS2-ERG</i> positive cores	30	100	16	100	46	46	46	100
Insertion	14	47	3	19	17	17	17	37
Deletion	16	53	13	81	29	29	29	63

* BIDMC = Beth Israel Deaconess Medical Center; UM = University of Michigan

Table 3 Morphological features by *TMPRSS2-ERG* fusion status in 100 patients' prostate cancer-positive biopsy cores

Morphological features	<i>TMPRSS2-ERG</i> status							P-value
	Negative			Positive			All	
	N	%	N	%	N	%	N	
All cores	54	100	46	100	100	100	100	--
Intraductal tumor spread	3	6	1	2	4	4	4	0.62
Cribriform growth pattern	4	7	11	24	15	15	15	0.03
Blue-tinged mucin	8	15	23	50	31	31	31	<0.01
Macronucleoli	16	30	25	54	41	41	41	0.02
Foamy gland morphology	4	7	0	0	4	4	4	0.12
Collagenous micromodules	0	0	4	9	4	4	4	0.04
Extraprostatic extension	1	2	0	0	1	1	1	--
Perineural invasion	6	11	9	20	15	15	15	0.27
Signet-ring cell features	4	7	0	0	4	4	4	0.12
Comedonecrosis	1	2	0	0	1	1	1	--
Glomerulations	3	6	2	4	5	5	5	1.0

Table 4

Number of morphological features and Gleason score by *TMPRSS2-ERG* fusion status of 100 patients' prostate cancer-positive biopsy cores.

	<i>TMPRSS2-ERG</i> status fusion						All		P-value
	Neg			Pos			N	%	
	N	%	N	%	N	%			
All cores	54	100	46	100	100	100			
Num. morphological features									<0.001
0	26	48	6	13	32	32			
1	13	24	13	28	26	26			
2	9	17	20	43	29	29			
3	5	9	6	13	11	11			
4	1	2	1	2	2	2			
Gleason Primary									0.53
3	41	76	33	72	74	74			
4	13	24	12	26	25	25			
5	-	-	1	2	1	1			
Gleason Secondary									0.46
3	25	46	24	52	49	49			
4	27	50	22	48	49	49			
5	2	4	0	0	2	2			
Gleason Sum									0.89
6	18	33	19	41	37	37			
7	30	56	19	41	49	49			
8	4	7	7	15	11	11			
9	2	4	1	2	3	3			

Table 5

Multivariable logistic regression of positive *TMPRSS2-ERG* fusion status among 100 patients' prostate cancer-positive biopsy cores.

Parameter	Odds Ratio	95% CI	P-value
PSA Density (ng/mL/cc)			0.050
Quartile 1, < 0.09	8.01	(1.8, 36.8)	
Quartile 2, 0.09 to <0.15	5.7	(1.3, 26.0)	
Quartile 3, 0.15 to <0.2	5.3	(1.1, 26.1)	
Quartile 4, 0.20	1 (ref)		
Cribriform growth pattern	9.4	(2.3, 38.6)	0.002
Blue-tinged mucin	11.6	(3.4, 39.4)	<.0001
Macronucleoli	5.3	(1.3, 22.1)	0.022

Abbreviations: PSA=prostate specific antigen; CI=confidence interval