Detection of TMPRSS2-ERG Fusion Transcripts and Prostate Cancer Antigen 3 in Urinary Sediments May Improve Diagnosis of Prostate Cancer

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Abstract

Purpose: Early detection of prostate cancer can increase the curative success rate for prostate cancer. We studied the diagnostic usefulness of TMPRSS2-ERG fusion transcripts as well as the combination of prostate cancer antigen 3 (PCA3) RNA and TMPRSS2-ERG fusion transcripts in urinary sediments after digital rectal examination (DRE).

Experimental Design: A total of 78 men with prostate cancer–positive biopsies and 30 men with prostate cancer–negative biopsies were included in this study. After DRE, the first voided urine was collected, and urinary sediments were obtained. We used semiquantitative reverse transcription-PCR (RT-PCR) analysis followed by Southern blot hybridization with a radiolabeled probe for the detection TMPRSS2-ERG fusion transcripts in these urinary sediments. A quantitative RT-PCR assay for PCA3 was used to determine the PCA3 score in the same sediments.

Results: TMPRSS2-ERG fusion transcripts can be detected in the urine after DRE with a sensitivity of 37%. In this cohort of patients, the PCA3-based assay had a sensitivity of 62%. When both markers were combined, the sensitivity increased to 73%. Especially in the cohort of men with persistently elevated serum prostate-specific antigen levels and history of negative biopsies, the high positive predictive value of 94% of TMPRSS2-ERG fusion transcripts could give a better indication which patients require repeat biopsies.

Conclusion: In this report, we used for the first time the combination of the prostate cancer–specific biomarkers TMPRSS2-ERG and PCA3, which significantly improves the sensitivity for prostate cancer diagnosis.

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths in the Western male population. It is estimated that prostate cancer will be diagnosed in 218,890 American men in 2007 (1). The high incidence of prostate cancer can be attributed to the increased life expectancy of Western males and the implementation of serum prostate-specific antigen (PSA) testing. Although the introduction of serum PSA in clinical practice has led to the detection of prostate cancer at a potentially curable stage, it has also led to the diagnosis of clinically insignificant prostate tumors. Another drawback of the serum PSA test is its low specificity, especially in the serum PSA gray zone of 3 to 15 ng/mL, resulting in a negative biopsy rate of 70% to 80%. Therefore, there is an urgent need for better biomarkers that can distinguish indolent from clinically significant prostate cancer and can reduce the number of unnecessary biopsies.

Differential gene expression analysis has been successfully used to identify prostate cancer–specific biomarkers by comparing malignant with nonmalignant prostate tissues. Recently, a new biostatistical method called cancer outlier profile analysis (COPA) was used to identify genes that are differentially expressed in a subset of prostate cancers (2). COPA identified strong outlier profiles for v-ets erythroblastosis virus E26 oncogene (ERG) and ets variant gene 1 (ETV1) in 57% of prostate cancer cases (3). This was in concordance with the results of a study where prostate cancer–associated ERG overexpression was found in 72% of prostate cancer cases (4). In >90% of the cases that overexpressed either ERG or ETV1, a fusion of the 5′-untranslated region of the prostate-specific and androgen-regulated transmembrane-serine protease gene (TMPRSS2) with these ETS family members was found. Recently, another fusion between TMPRSS2 and an ETS family member has been described, the TMPRSS2-ETV4 fusion, although this fusion is sporadically found in prostate cancers

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(5). These fusions can explain the aberrant androgen-dependent overexpression of ETS family members in subsets of prostate cancer because TMPRSS2 is androgen regulated (3). The discovery of the TMPRSS2-ERG fusion transcript and the fact that ERG is the most frequently overexpressed proto-oncogene described in malignant prostate epithelial cells suggests its role in prostate tumorigenesis. As such, it may have impact not only on improved diagnosis but also on the treatment of prostate cancer.

For the diagnosis of prostate cancer, ideally, biomarkers should be detectable using noninvasive methods, e.g., by means of a blood or urine test. Recently, attention was focused on prostate cancer antigen 3 (PCA3), a noncoding RNA that is prostate specific and highly overexpressed in prostate cancer (6). Independent studies have shown that a PCA3-based urine test can improve the specificity in prostate cancer diagnosis and could thus aid in reducing biopsies (7, 8). Because prostate cancer is a heterogeneous disease, the use of a panel of markers can further improve the diagnosis of this disease.

A recent study done on a cohort of 19 prostate cancer patients showed that the detection of TMPRSS2-ERG fusion transcripts in the urine of prostate cancer patients is feasible (9). For the first time, we report on the diagnostic usefulness of TMPRSS2-ERG fusion transcripts in combination with PCA3 in urinary sediments in patients who were to undergo prostate biopsies based on serum PSA levels ≥3 ng/mL and/or an abnormal digital rectal examination (DRE).

Materials and Methods

Collection of urine samples after DRE. In the urological outpatient clinic, the first voided urine after DRE was collected prospectively from men who were admitted for prostate biopsies based on serum PSA levels ≥3 ng/mL and/or abnormal DRE. All men had received study information, and they had signed their informed consent. In this study, a total of 108 patients were included, of whom 78 (72%) had prostate cancer upon biopsy. This patient population does not represent the typical patient population having a prostate biopsy based on elevated serum PSA levels in which the percentage of prostate cancer positive men is about 30%. The serum PSA values ranged from 1.1 to 1,619.0 ng/mL.

The DRE was done by applying firm pressure to the prostate from the base to apex and from the lateral to the median line of each lobe. The men were asked to void, and the first 30 mL of urine was collected. Following urine collection, the urologist measured prostate volume by transrectal ultrasonography and did the prostate biopsy according to a standard protocol (at least three biopsies from the left peripheral zone, at least three biopsies from the right peripheral zone, one biopsy from the left transition zone, and one biopsy from the right transition zone, plus additional biopsies from other areas when suspicious for prostate cancer). All biopsy cores were examined for the presence of prostate cancer by a pathologist.

After collection, the urine samples were immediately cooled on ice. Upon centrifugation at 4°C and 700 × *g* for 10 min, urinary sediments were obtained. These urinary sediments were washed twice with ice-cold PBS. Upon centrifugation at 4°C and 700 × *g* for 10 min, the sediments were snap frozen in liquid nitrogen and stored at -70°C. The urinary sediments were added to 20 μg of *Escherichia coli* tRNA as a carrier (Roche Diagnostics), and total RNA was extracted using the TRIzol reagent (Invitrogen). The PCA3 score was determined in all urinary sediments as described previously (10, 11).

Reverse transcription-PCR analysis. Total RNA was DNase treated and used for reverse transcription with SuperScript II RNase H$^-$ Reverse Transcriptase (Invitrogen). The cDNA synthesis was done in a 30-μL reaction volume, which contained 4 μL of RNA, 300 units of reverse transcriptase, 1× first-strand buffer, 10 mmol/L of DTT, 1 mmol/L of each deoxynucleotide triphosphate (dNTP), and 1 nmol of random hexamers.

Primer pairs were designed with a forward primer in exon 1 of TMPRSS2, T2-F1: 5′-CGCGAGCTAAGCAGGAG-3′ (position 1-17, GenBank database accession number NM_005656), and reverse primers in exon 4 of ERG, ERG-R4: 5′-GTCCATAGTCGCTGGAGGAG-3′, and exon 6 of ERG, ERG-R6: 5′-CCATATTCTTTCACCGCCCACTCC-3′ (respectively, positions 315-334 and 636-659, GenBank database accession number NM_004449). For each sample, a control PCR was done with primers for the housekeeping gene β2-microglobulin B2M-for: 5′-AGCAGAGAATGGAAAGTCAAA-3′ and B2M-rev: 5′-TGCTGCTTACATGTCTCG-3′ (respectively, positions 162-182 and 409-426, GenBank database accession number NM_004048). About 2 μL of cDNA sample was amplified in a 25-μL PCR reaction containing 25 pmol of each primer, 150 μmol/L of each dNTP (Roche Diagnostics), 0.02 units of SuperTaq polymerase (HT Biotechnologie LTD) in buffer containing 1.5 mmol/L magnesium chloride, 10 mmol/L Tris-HCl (pH, 8.3), 50 mmol/L potassium chloride, and 0.1% Triton X-100. PCR amplification for TMPRSS2-ERG transcripts was done on a Thermal Cycler (Eppendorf AG) as follows: 95°C for 2 min followed by 40 cycles at 94°C for 45 s, 64°C for 45 s, and 72°C for 45 s, followed by a final extension of 10 min at 72°C. The same conditions were used for PCR amplification of β2-microglobulin with the exception that 26 amplification cycles were done and an annealing temperature of 60°C was used.

Detection of the PCR products. Amplified PCR products were separated on a 2% agarose gel and blotted onto Hybond-N+ membranes (GE Healthcare) using blot buffer containing 0.5 mol/L NaOH and 1.5 mol/L NaCl. A probe was constructed by cloning the TMPRSS2-ERG (T2-F1/ERG-R4) PCR fragment into the pCR-Blunt vector (Invitrogen), verified by sequence analysis and labeled by random prime labeling using [α-^{32}P]dATP, 10 mCi/mL, 3,000 Ci/mmol (GE Healthcare).

Hybridization of the Southern blots was done as has been described previously (12). Briefly, the blots were preincubated in hybridization buffer [7% SDS; 1% bovine serum albumin; 0.5 mol/L sodium phosphate buffer (pH, 7.4); 1 mmol/L EDTA; 100 μg/mL salmon sperm DNA] for 1 h at 65°C. The radiolabeled probe was added to a maximum of 1 × 10^6 cpm/mL, and the blots were hybridized for 3 h at 65°C.

Blots were washed at high stringency with buffers containing 1% SDS, 1 mmol/L EDTA, and 0.5 mol/L sodium phosphate (wash buffer 1) and 0.1 mol/L sodium phosphate (wash buffer 2) for 15 min each at 65°C. Specific hybridization signals were visualized by autoradiography using X-ray films (GE Healthcare).

Statistical analysis. The Pearson χ^2 test was used to test the hypothesis that the presence of TMPRSS2-ERG fusion transcripts in urinary sediments is related to the Gleason score determined in the prostate biopsy specimen.

Results

TMPRSS2-ERG fusion transcripts can be detected in urine after DRE. Urinary sediments were obtained after DRE from 78 men with prostate cancer–positive biopsies and 30 men with prostate cancer–negative biopsies. All 108 urine samples were positive for the housekeeping gene β2-microglobulin, used as a control for cDNA synthesis, indicating that all samples contained cells (data not shown). All samples were positive for PSA mRNA, quantitatively determined in all of these specimens as described previously, indicating that cells of prostate origin were present in these urinary sediments (data not shown).

After reverse transcription-PCR (RT-PCR) amplification of TMPRSS2-ERG fusion transcripts, a heterogeneous pattern of

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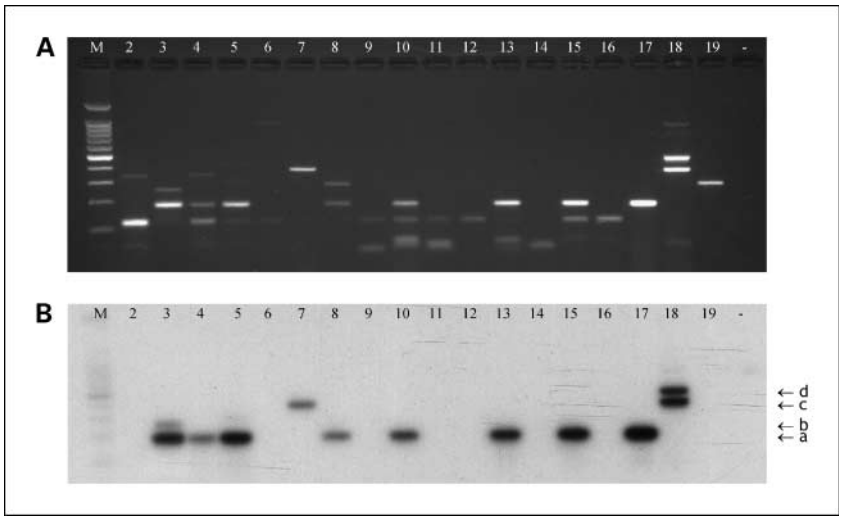

Fig. 1. Detection of TMPRSS2-ERG fusion transcripts in urinary sediments after DRE. *A,* RT-PCR analysis on RNA extracted from 18 urinary sediments with a forward primer located in TMPRSS2 exon 1 and a reverse primer located in ERG exon 4. The PCR products were separated by agarose gel electrophoreses. Lane 1, M, 100 bp DNA ladder (Promega); lanes 2 to 19, urinary sediments; lane 20, negative control. *B,* autoradiogram of the Southern blot from agarose gel shown in (*A*), hybridized with a ^{32}P-labeled probe specific for TMPRSS2-ERG fusion transcripts. The most common fusion transcript from TMPRSS2 exon 1 to ERG exon 4 can be seen in lanes 3–5, 8, 10, 13, 15, and 17 (a, 180 bp). In lane 3, a variant fusion transcript is present of TMPRSS2 exon 2 and ERG exon 4 (b, 251 bp). A fusion between TMPRSS2 exon 1 and ERG exon 2 can be seen in lanes 7 and 18 (c, 368 bp). Lane 18 contains the same fusion transcript as in lane 7, together with a fusion transcript of TMPRSS2 exon 2 and ERG exon 2 (d, 439 bp). The nature of all fusion transcripts was confirmed by sequence analysis.

PCR products was observed on agarose gel electrophoresis (Fig. 1A). After Southern blotting and hybridization with a radiolabeled probe specific for TMPRSS2-ERG fusion transcripts containing TMPRSS2 exon 1 or ERG exon 4, only specific bands remained visible (Fig. 1B). Bidirectional sequence analysis confirmed that the PCR products that did not hybridize to the probe were not TMPRSS2-ERG–related, and that the PCR products that did hybridize represent the actual TMPRSS2-ERG fusion transcripts (Figs. 1B and 2).

When we did semiquantitative RT-PCR on RNA extracted from 29 primary prostate cancer tissue specimens, we found that TMPRSS2-ERG fusion transcripts were detected in 59% of the cases (data not shown). The urinary sediments of 29 of the 78 (37%) prostate cancer patients and 2 of the 30 men with negative biopsies (7%) harbored TMPRSS2-ERG fusion transcripts (Table 1).

The transcript TMPRSS2 exon 1 fused with ERG exon 4 was found in 27 of 31 (87%) fusion transcript–positive cases (Table 2). In the urinary sediments of three prostate cancer patients, this transcript was found together with variant fusion transcripts. These variant fusion transcripts are TMPRSS2 exon 1 fused with ERG exon 2 and TMPRSS2 exon 2 fused with ERG exon 4. In four prostate cancer patients, only variant fusion transcripts were found: TMPRSS2 exon 1 fused with ERG exon 2 or 3, and TMPRSS2 exon 2 fused with ERG exon 2 or 4 (Table 2). Using the Pearson's χ^2 test, no relationship was found between the presence of TMPRSS2-ERG transcripts in urinary sediments and Gleason score in prostate biopsy specimens (two-sided $P = 0.511$; Table 3). In the total cohort of patients, the analysis for TMPRSS2-ERG fusion transcripts in urinary sediments had a sensitivity of 37%, a specificity of 93%, a negative predictive value of 36%, and a positive predictive value of 94% for the detection of prostate cancer (Table 1).

TMPRSS2-ERG and PCA3 in prostate cancer diagnosis. To evaluate whether TMPRSS2-ERG fusion transcript analysis could be of additional value to the PCA3 test, the PCA3 score was determined as described previously (10). In the absence of an arbitrary cutoff value, we determined from a Dutch multicenter study a cutoff value of 58 for prostate cancer diagnosis based on a receiver-operating characteristic curve (11). In the study described here, samples with higher values than this cutoff were considered as positive PCA3 test results. A total of 48 urinary sediments of 78 men with prostate cancer had a positive PCA3 test, indicating a sensitivity of 62% (Table 4A).

In the total cohort of patients, the urinary sediments of 21 men were found positive for both PCA3 and TMPRSS2-ERG translocations (Table 4B). Twenty of these men (95%) had prostate cancer upon biopsy (Table 4A). The urinary sediments of 21 prostate cancer patients (27%) were negative for both markers. In the total cohort, 28 of the 41 men (44%) who had a positive PCA3 test but were negative for TMPRSS2-ERG fusion transcripts had prostate cancer upon biopsy. A total of 9 of the 10 men (90%) who had a negative PCA3 test but were positive for TMPRSS2-ERG fusion transcripts had prostate cancer. These data are summarized in Table 4A and B.

The PCA3 test alone detected 48 prostate cancer patients. Combined with TMPRSS2-ERG translocations, the number of prostate cancers detected increased to 57. This indicates that the sensitivity of the combined tests is 73%.

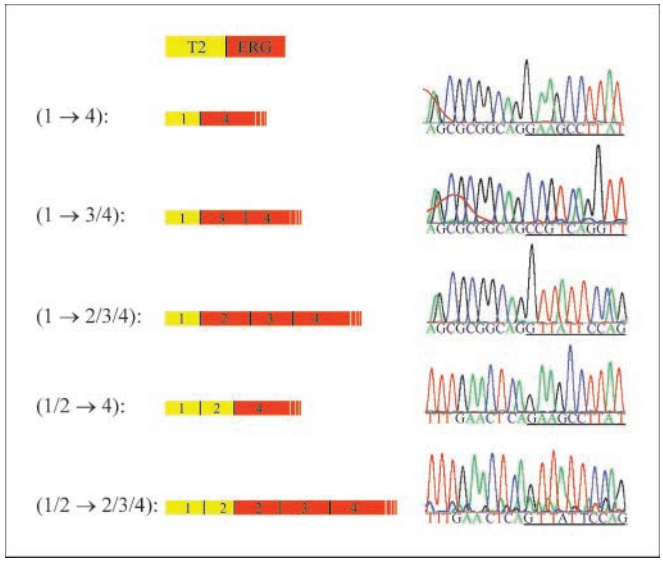

Fig. 2. Partial chromatogram of sequence analysis of the TMPRSS2-ERG fusion transcripts found in the urinary sediments. In the chromatogram, the ERG sequence is underlined.

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Table 1. Diagnostic performance of TMPRSS2-ERG fusion transcripts in urinary sediments

	PCa	NPr			
TMPRSS2-ERG ⊕	29	2	31	Sensitivity	0.37
TMPRSS2-ERG ⊖	49	28	77	Specificity	0.93
	78	30	108	NPV	0.36
				PPV	0.94

Abbreviations: PCa, prostate cancer–positive biopsies. NPr, negative biopsies.

Discussion

Gene fusions have been predominantly found in hematologic malignancies (leukemias and lymphomas) and soft-tissue tumors (Ewing's sarcomas) and have been far less frequently described in epithelial cancers, which account for 80% of cancer-related deaths (2). Recently, fusions of the 5′-untranslated region of TMPRSS2 with the ETS transcription factors ERG, ETV1, and ETV4 have been reported in prostate cancer, which is one of the most common epithelial tumor types (3, 5). A small study on 19 patients with prostate cancer showed that TMPRSS2-ERG fusion transcripts could be detected in urinary sediments with a sensitivity of 42% (9). In this study, urinary sediments obtained from 108 men who were indicated for prostate biopsies based on serum PSA levels ≥3 ng/mL and/or abnormal DRE, TMPRSS2-ERG fusion transcripts were detected in 37% of men with prostate cancer–positive biopsies.

In previous studies on RNA extracted from radical prostatectomy tissue samples, the frequency of TMPRSS2-ERG fusion transcripts, determined either by fluorescence *in situ* hybridization or a RT-PCR–based approach, was 40% to 59% (3, 13–16). In our cohort of 29 radical prostatectomy specimens, TMPRSS2-ERG fusion transcripts were detected in 59% of the cases (data not shown). These observations suggest that ~50% of the prostate cancers harbor TMPRSS2-ERG fusion transcripts.

There can be several explanations for the lower frequency of TMPRSS2-ERG fusion transcripts observed in urinary sediments compared with radical prostatectomy tissue samples. One explanation could be that most of the previous studies are based on radical prostatectomy tissue specimens. For the detection of TMPRSS2-ERG fusion transcripts in the urine, we are dependent on the ability of prostate cancer cells to be mobilized in the urine. To gain insight whether the urinary sediments contained prostate cancer cells, we compared the TMPRSS2-ERG results with PCA3 results in these specimen. PCA3-based assays using different amplification methods for the detection of PCA3 RNA transcripts have shown their diagnostic value (7, 8, 10, 11). Because PCA3 is strongly overexpressed in more than 95% of primary prostate cancer specimens, only 5% of cancers should be false negative (6, 17). We found a higher false-negative rate of 27% for both markers; it suggests that this is due to the fact that no or very few cancer cells were released into the urine upon DRE. This is in agreement with the hypothesis of Tinzl et al. (7) that false-negative

Table 2. Overview of TMPRSS2-ERG fusion transcripts determined in urinary sediments

Patient	Serum PSA, ng/mL	PCA3 test result	Gleason score in biopsies	TMPRSS2-ERG fusion transcripts
17	10.4	-	No malignancy	1 → 4
47	1.1	+	No malignancy	1 → 4
70	6.4	+	2 + 3 = 5	1 → 4
89	4.7	-	2 + 3 = 5	1 → 4
4	7.6	+	3 + 3 = 6	1 → 4
44	7.3	+	3 + 3 = 6	1 → 4
52	3.4	+	3 + 3 = 6	1 → 4
67	5.0	+	3 + 3 = 6	1 → 4
79	17.6	-	3 + 4 = 7	1 → 4
88	7.4	+	3 + 4 = 7	1 → 4
90	11.4	-	3 + 4 = 7	1 → 4
95	96.7	-	3 + 4 = 7	1 → 4
96	22.5	-	3 + 4 = 7	1 → 4
100	4.7	+	3 + 4 = 7	1 → 4
61	6.6	-	4 + 3 = 7	1 → 4
106	11.8	-	4 + 3 = 7	1 → 4
110	122.0	+	4 + 3 = 7	1 → 4
2	73.6	+	4 + 4 = 8	1 → 4
46	82.1	+	5 + 3 = 8	1 → 4
71	85.6	+	4 + 5 = 9	1 → 4
104	12.6	+	4 + 5 = 9	1 → 4
103	103.0	+	5 + 4 = 9	1 → 4
109	12.4	+	5 + 4 = 9	1 → 4
54	16.0	+	5 + 5 = 10	1 → 4
68	9.1	-	3 + 3 = 6	1/2 → 4
62	4.9	+	3 + 3 = 6	1 → 2/3/4
15	9.8	+	3 + 4 = 7	1 → 4 + 1 → 2/3/4
27	16.3	+	3 + 2 = 5	1 → 4 + 1/2 → 4
40	8.3	+	3 + 5 = 8	1 → 4 + 1/2 → 4
43	7.6	-	5 + 4 = 9	1 → 2/3/4 + 1 → 3/4
102	7.1	+	4 + 5 = 9	1 → 2/3/4 + 1/2 → 2/3/4

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samples may represent a subgroup of prostate tumors that have less tendency to invade in the prostate ductal system and, thus, shed less cells in the urine. An alternative explanation could be that we restrict ourselves to the fusion transcripts that were described most frequent containing TMPRSS2 exon 1 and ERG exon 4 (14). In the urinary sediments, the most frequently observed fusion transcript was TMPRSS2 exon 1 fused with ERG exon 4 (87%) either alone or in combination with other fusion types (Table 2 and Fig. 2). This is comparable with the findings of Wang et al. (14), who detected this fusion transcript also in 86% of the cases. Several studies have reported on the expression of variant transcripts in which TMPRSS2 exon 1 is fused with ERG exon 5 (14, 18). In individual samples, this fusion transcript usually occurs together with the most commonly expressed transcript (TMPRSS2 exon 1 and ERG exon 4). Using a reverse primer in ERG exon 6, we confirmed for 29 radical prostatectomy tissue specimens (data not shown) that the transcript of TMPRSS2 exon 1 fused with ERG exon 5 was indeed found together with the most commonly expressed transcript. Therefore, it did not lead to the detection of additional prostate cancers. These findings indicate that the exclusion of the transcript TMPRSS2 exon 1 fused with ERG exon 5 would not be the major reason for the observed lower sensitivity.

Recently, there have been reports on the prognostic value of TMPRSS2-ERG fusion transcripts. The Gleason scoring system, in which primary and secondary cancer growth patterns are identified and summed, is regarded as one of the most powerful prognosticators in prostate cancer. It was shown that the expression of TMPRSS2-ERG fusion transcripts is associated with higher Gleason scores (19, 20). Moreover, a statistically significant association between TMPRSS2-ERG fusion transcripts and prostate cancer–specific death was found (20). In our study, we could not confirm that the urine-based TMPRSS2-ERG analysis is associated with a higher Gleason score (P = 0.511). An explanation could be that we used prostate biopsy tissue specimen to determine the Gleason score, whereas in other studies, radical prostatectomy tissue specimens or TURP material was used. The fact that prostate cancer is a heterogeneous disease can result in undergrading of the Gleason score in biopsies. It is therefore most likely that the Gleason score of many of these cancers will be upgraded at surgery. Therefore, it would be interesting to compare these results with the final Gleason score after radical surgery.

Table 3. Prognostic value of TMPRSS2-ERG fusion transcripts in urinary sediments of prostate cancer patients

	TMPRSS2-ERG negative, *n* (%)	TMPRSS2-ERG positive, *n* (%)
Number of cases	49	29
Gleason score <7	18 (37)	9 (31)
Gleason score =7	20 (41)	10 (34)
Gleason score >7	11 (22)	10 (34)

NOTE: No significant relationship was found between the presence of TMPRSS2-ERG fusion transcripts in urinary sediments and Gleason score determined in prostate biopsies (P = 0.511).

Table 4.

A. Concordance analysis of TMPRSS2-ERG versus PCA3 in prostate cancer patients

	PCA3 ⊕	PCA3 ⊖	
TMPRSS2-ERG ⊕	20	9	29
TMPRSS2-ERG ⊖	28	21	49
	48	30	78

B. Concordance analysis of TMPRSS2-ERG versus PCA3 in all patients

	PCA3 ⊕	PCA3 ⊖	
TMPRSS2-ERG ⊕	21	10	31
TMPRSS2-ERG ⊖	41	36	77
	62	46	108

Because prostate cancer is a heterogeneous disease, it becomes clear that a combination of markers will become important in early prostate cancer diagnosis (21). The urinary sediments of prostate cancer patients that were found positive for PCA3 but negative for TMPRSS2-ERG fusion transcripts and vice versa support this idea. Nine prostate cancers could be detected in case the negative PCA3 urine test was combined with TMPRSS2-ERG fusion transcripts (Table 2). The combination of both markers remarkably increased the sensitivity for the detection of prostate cancer from 62% (PCA3 test alone) to 73%.

The combination of both markers could be of special value in men who have persistently elevated serum PSA values, but a history of negative biopsies. Several men included in the study published by Hessels et al. (10) who had a positive PCA3 test but negative biopsies were shown to have prostate cancer upon repeated biopsies.[3] In this study, there are two men with negative biopsies who were positive for TMPRSS2-ERG fusion transcripts. One of them is also positive for PCA3. Because the positive predictive value (PPV) of TMPRSS2-ERG fusion transcript analysis is 94%, this indicates that these two men have a probability of 94% of having prostate cancer. Because the urinary sediments in this study were collected prospectively, follow-up data on these evaluated patients are rather short. However, the results of this study suggest that if both tests are combined, a better indication can be given which patients need a repeat biopsy and, as such, could aid the diagnosis of prostate cancer as a reflex test to serum PSA. Follow-up data will show in time whether PCA3-based and TMPRSS2-ERG–based diagnostics can detect prostate cancers before prostate cancer is diagnosed by biopsy.

In summary, we show that the noninvasive detection of TMPRSS2-ERG fusion transcripts is feasible in urinary sediments obtained after DRE. This is the first report where two novel prostate cancer–specific biomarkers are combined for the diagnosis of prostate cancer. The combination of TMPRSS2-ERG fusion transcripts and PCA3 RNA transcripts remarkably improves the sensitivity without compromising specificity for the detection of prostate cancer. This combination could be of special value in men who have persistently elevated serum PSA

[3] Unpublished data.

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values but a history of negative biopsies. The high positive predictive value of TMPRSS2-ERG fusion transcript analysis could aid the clinician to decide which patient needs a repeat biopsy.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Evidence of recurrent gene fusions in common epithelial tumors. Trends Mol Med 2006;12:529–36.
3. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
4. Petrovics G, Liu A, Shaheduzzaman S, et al. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene 2005;24:3847–52.
5. Tomlins SA, Mehra R, Rhodes DR, et al. TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. Cancer Res 2006;66:3396–400.
6. Bussemakers MJ, van Bokhoven A, Verhaegh GW, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res 1999;59:5975–9.
7. Tinzl M, Marberger M, Horvath S, Chypre C. DD3PCA3 RNA analysis in urine—a new perspective for detecting prostate cancer. Eur Urol 2004;46:182–6.
8. Fradet Y, Saad F, Aprikian A, et al. uPM3, a new molecular urine test for the detection of prostate cancer. Urology 2004;64:311–5.
9. Laxman B, Tomlins SA, Mehra R, et al. Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. Neoplasia 2006;8:885–8.
10. Hessels D, Klein Gunnewiek JM, van Oort I, et al. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003;44:8–15.
11. van Gils MP, Hessels D, van Hooij O, et al. The time-resolved fluorescence-based PCA3 test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. Clin Cancer Res 2007;13:939–43.
12. Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci U S A 1984;81:1991–5.
13. Yoshimoto M, Joshua AM, Chilton-Macneill S, et al. Three-color FISH analysis of TMPRSS2/ERG fusions in prostate cancer indicates that genomic microdeletion of chromosome 21 is associated with rearrangement. Neoplasia 2006;8:465–9.
14. 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.
15. Perner S, Demichelis F, Beroukhim R, et al. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res 2006;66:8337–41.
16. Cerveira N, Ribeiro FR, Peixoto A, et al. TMPRSS2-ERG gene fusion causing ERG overexpression precedes chromosome copy number changes in prostate carcinomas and paired HGPIN lesions. Neoplasia 2006;8:826–32.
17. de Kok JB, Verhaegh GW, Roelofs RW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res 2002;62:2695–8.
18. Clark J, Merson S, Jhavar S, et al. Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. Oncogene 2006;26:2667–73.
19. Rajput AB, Miller MA, De LA, et al. Frequency of the TMPRSS2:ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol 2007. Epub ahead of print.
20. Demichelis F, Fall K, Perner S, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007. Epub ahead of print.
21. Hessels D, Verhaegh GW, Schalken JA, Witjes JA. Applicability of biomarkers in the early diagnosis of prostate cancer. Expert Rev Mol Diagn 2004;4:513–26.

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