

# Molecular genetic analyses of the *TMPRSS2-ERG* and *TMPRSS2-ETV1* gene fusions in 50 cases of prostate cancer

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**Abstract.** Recently, gene fusions between the androgen responsive gene *TMPRSS2* and members of the ETS-family of DNA-binding transcription factor genes were found in prostate cancer. Recurrent fusions were identified between the 5'-noncoding region of *TMPRSS2* and *ERG*, or less frequently *ETV1* or *ETV4*, resulting in overexpression of normal or truncated ETS-proteins. Herein, we have analyzed a series of 50 prostate cancer samples for expression of *TPRSS2-ERG* and *TMPRSS2-ETV1* fusion transcripts. RT-PCR analysis revealed *TMPRSS2-ERG* fusion transcripts in 18 of the 50 tumors (36%). None of the tumors expressed a *TMPRSS2-ETV1* fusion. Our findings show that the *TMPRSS2-ERG* fusion is common in prostate cancer and that the related *TMPRSS2-ETV1* fusion is very rare. However, the frequency of *ERG*-fusions in the present study is somewhat lower than previously observed, indicating heterogeneity with regard to expression of ETS-gene fusions in subsets of prostate cancers. Moreover, clinical follow-up studies showed a clear tendency that fusion-positive tumors were associated with lower Gleason grade and better survival than fusion-negative tumors. Our findings suggest that *ERG* gene fusions might be of prognostic significance in prostate cancer.

## Introduction

In developed countries, prostate cancer is the second most commonly diagnosed cancer and the third most common cause of cancer death among men (1,2). In addition to patient's age and race, a positive family history is probably the strongest known risk factor for prostate cancer (3). In its clinical form, prostate cancer is diagnosed as local or advanced, and treatments range from surveillance, to radical local treatment,

or androgen deprivation therapy. Androgen deprivation reduces clinical symptoms in ~70-80% of patients with advanced prostate cancer, but most tumors relapse within two years to an androgen independent state (4), and so far it has not been possible to cure patients with metastatic disease. In order to find new therapeutic pathways, the genetic mechanisms behind prostate cancer initiation and tumor progression towards androgen independence and metastases need to be thoroughly elucidated.

An important breakthrough in the search for novel pathogenetic mechanisms in prostate cancer was recently reported when fusion oncogenes involving members of the ETS-family of transcription factor genes were identified in prostate cancer (5,6). Recurrent fusions were found between the 5'-noncoding region of the *TMPRSS2* gene and the *ERG*, *ETV1* or *ETV4* genes, resulting in overexpression of normal or truncated ETS-proteins. The fusions were found in 45-79% of prostate cancer samples. The *TMPRSS2-ERG* fusion is by far the most common fusion type followed by *TMPRSS2-ETV1* and *TMPRSS2-ETV4*, in that order of frequency.

The *ERG*, *ETV1* and *ETV4* genes all encode transcription factors containing a highly conserved, sequence-specific DNA-binding domain, the so-called ets domain (7). For reasons that remain unclear ETS-family genes are frequently targeted by chromosomal rearrangements in various human malignancies, resulting in overexpression of oncogenic ETS-gene fusions (7). The *TMPRSS2* gene encodes an androgen regulated, type II transmembrane-bound serine protease that is highly expressed in normal prostate tissue as well as in neoplastic prostate epithelium (8,9). However, the *in vivo* biological activities of *TMPRSS2* are unclear and recent studies have shown that mice lacking the *Tmprss2*-encoded protease are viable and fertile and have no detectable abnormalities, suggesting functional redundancy involving one or more of the type II transmembrane serine proteases or other serine proteases (10). The molecular consequences of the ETS-gene fusions in prostate cancer are unclear. *In vitro* experiments have suggested that the ectopic expression of ETS-family members is mediated by androgen-responsive elements in the *TMPRSS2* promoter (5).

To obtain additional information about the incidence and potential clinical significance of ETS gene fusions in prostate cancer we have screened a series of 50 prostate cancer specimens for expression of the *TMPRSS2-ERG* and *TMPRSS2-ETV1* gene fusions. RT-PCR analysis revealed

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*TMPRSS2-ERG* fusion transcripts in 36% (18/50) of the tumors. None of the tumors expressed the *TMPRSS2-ETV1* fusion. Clinical follow-up studies showed a clear tendency that fusion-positive tumors were associated with lower Gleason grade and better survival compared with fusion-negative tumors.

## Materials and methods

**Patients.** Prostate tumor biopsies were obtained from 50 patients presenting with clearly palpable tumors of the prostate at the outpatient Urology Clinic at Sahlgrenska University Hospital in Gothenburg, Sweden from April 2000 to February 2003. The median age of the patients was 76 years (range 53.7-86.2 years). Local tumor stage was determined by digital rectal examination. A transrectal ultrasound (TRUS) investigation was subsequently performed and 2-5 ultrasound-guided diagnostic needle biopsies were taken from the tumor under the protection of per oral antibiotic prophylaxis. Biopsies were fixed in formaldehyde according to routine procedures. At the same time, two additional TRUS-guided biopsies were taken from the same part of the tumor as the diagnostic biopsies. These biopsies were instantly frozen in liquid nitrogen and stored at -70°C. All patients had histologically confirmed prostatic carcinomas with a Gleason score ranging from 3+3=6 to 5+5=10. The presence of cancer in the frozen biopsies was not confirmed histologically, but the probability of them containing cancer was very high since in 39 patients, cancer was found in all diagnostic biopsies and in the 11 remaining patients, cancer was found in more than half of the biopsies. There were 16 T2, 28 T3, and 6 T4 tumors. M-stage was determined by radionuclide scanning and signs of distant metastases (M1) were found in 26 cases. A negative radionuclide scanning (M0) was found in 12 cases and in 12 cases no scanning was performed (Mx). All patients received endocrine treatment either by subcutaneous injection of a GnRH analog once every 3 months or by surgical castration. A pretreatment PSA level was established prior to the first visit with the urologist and following the onset of treatment, PSA was monitored regularly every three months. In April 2006, 31 patients (62%) had died. The cause of death for these patients was determined by examination of death certificates. Ethics approval for use of these samples was obtained from the Ethics Committee of the Sahlgrenska University Hospital, Göteborg, Sweden (No. 667-05).

**Statistical analyses.** Kaplan-Meier analyses were performed to evaluate progression-free survival and cause-specific survival. The  $\chi^2$ -test was used to evaluate possible differences between fusion-positive and fusion-negative patients with regard to Gleason score and time to PSA progression.

**Isolation of RNA, RT-PCR, and nucleotide sequence analyses.** Total RNA was extracted from frozen tumor biopsies using the RNeasy micro kit (Qiagen). For cDNA synthesis, 1  $\mu$ g of total RNA was reverse-transcribed using the Superscript™ First-strand synthesis system according to the manufacturer's manual (Invitrogen). As a control for intact RNA and cDNA, RT-PCR reactions for expression of the housekeeping gene *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) was

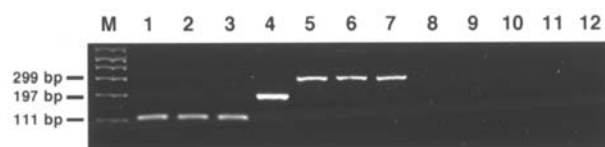


Figure 1. Expression of *TMPRSS2-ERG* fusion transcripts in prostate cancer. RT-PCR analysis using the primers *TMPRSS2-301F* and *ERG-304R* revealed three types of chimeric transcripts, consisting of *TMPRSS2* exon 1 fused to either *ERG* exon 4 (lanes 1-3), *ERG* exon 3 (lane 4) or *ERG* exon 2 (lanes 5-7). Lanes 8-11 represent four fusion-negative prostate tumors. The control lane (lane 12) represents a PCR reaction with fusion-specific primers, but without cDNA template. Sizes of *TMPRSS2-ERG* fusion transcripts are indicated.

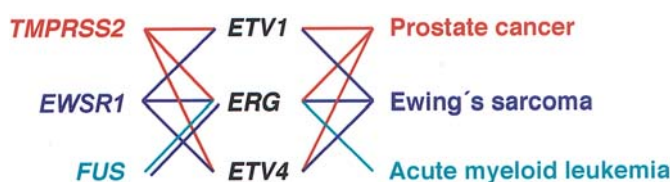


Figure 2. Fusion oncogenes involving the ETS-family members *ERG*, *ETV1* and *ETV4* in human cancer. In all fusions depicted, the *TMPRSS2*, *EWSR1* and *FUS* genes are disrupted and their 5'-parts fused to the 5'-ends or central parts of ETS-genes. Note that the *FUS-ERG* fusion has been detected in both Ewing's sarcoma and acute myeloid leukemia, whereas the other fusions appear to be tumor type specific.

performed on all cDNAs using the primers *GAPDH-48F* 5'-GTGAAGGTCGGAGTCAACG-3' and *GAPDH-48R* 5'-GGTG AAGACGCCAGTGGACTC-3'.

The *TMPRSS2-ERG* fusion transcript was amplified with the primers *TMPRSS2-301F* 5'-CAGGAGGCGGAGGCGGA-3' and *ERG-304R* 5'-GTAGGCACACTCAAACAACGACTGG-3' or *TMPRSS2-301F* 5'-CAGGAGGCGGAGGCGGA-3' and *ERG-305R* 5'-GGCGTTGTAGCTGGGGGTGAG-3' (5). The *TMPRSS2-ETV1* fusion transcript was amplified with the primers *TMPRSS2-301F* 5'-CAGGAGGCGGAGGCGGA-3' and *ETV1-302R* 5'-CAGGCCATGAAAGCCAAACTT-3' or *TMPRSS2-301F* 5'-CAGGAGGCGGAGGCGGA-3' and *ETV1-303R* 5'-TTGTGGTGGGAAGGGATGTTT-3' (5). PCR products were gel purified and sequenced using the ABI PRISM® BigDye™ terminator cycle sequencing ready reaction v2.0 kit (Applied Biosystems, Foster City, CA) in an ABI Prism 310 genetic analyzer (Applied Biosystems). The resulting sequences were analysed using the bioinformatics tools provided by the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov>).

## Results and Discussion

By using RT-PCR we screened a series of 50 prostate cancer specimens for expression of the *TMPRSS2-ERG* and *TMPRSS2-ETV1* gene fusions. Chimeric transcripts corresponding to *TMPRSS2-ERG* fusions were detected in 18 of the 50 tumors (36%). In contrast, none of the tumors expressed *TMPRSS2-ETV1* fusion transcripts. Three types of *TMPRSS2-ERG* chimeric transcripts were detected, consisting of *TMPRSS2* exon 1 fused to either *ERG* exon 4 (14 cases),

**SPANDIDOS** Time to PSA progression, metastases and Gleason correlation to *TMPRSS2-ERG* status.

		<i>TMPRSS2-ERG</i> positive patients	<i>TMPRSS2-ERG</i> negative patients
Time to PSA progression	≤1 year	3/14 (21%)	11/14 (79%)
	>1 year	14/34 (41%)	20/34 (59%)
Metastases	Yes	9/26 (35%)	17/26 (65%)
	No	5/12 (42%)	7/12 (58%)
Gleason score	≤3+4	8/14 (57%)	6/14 (43%)
	≥4+3	10/36 (28%)	26/36 (72%)

*ERG* exon 2 (four cases; GenBank accession no. DQ831522) or *ERG* exon 3 (one case; GenBank accession no. DQ831521) (Fig. 1). *TMPRSS2* exon 1 - *ERG* exon 3 represents a novel fusion transcript. All fusion transcripts were confirmed by using a second combination of PCR primers as well as by nucleotide sequence analysis of the PCR products.

The present investigation confirms and extends previous data showing that the *TMPRSS2-ERG* fusion is recurrent in prostate cancer (5,11). However, the frequency of *ERG*-fusions in our study is somewhat lower than that recently reported, indicating heterogeneity with regard to expression of ETS-gene fusions in subsets of prostate cancers. Thus, Tomlins *et al* in their original study (5) found *TMPRSS2-ERG* fusion transcripts in 19 of 42 (45%) samples of localized (32 samples) and metastatic (10 samples) prostate cancer and a *TMPRSS2-ETVI* fusion in one sample (2.4%). In the same study, FISH analysis of an independent tissue microarray containing core biopsies from 13 cases of localized and 16 cases of metastatic prostate cancer revealed evidence of *ERG*- or *ETVI*-fusions in 23 of the 29 cases (79.3%). A similar frequency of *ERG*-fusions was recently also reported in a small series of 18 prostate cancer samples (11). As pointed out by Tomlins *et al* the frequency of ETS-gene fusions in their study might have been overestimated due to the fact that the FISH assay used can also detect other *ERG* rearrangements (5). Similarly, we cannot exclude the possibility that the frequency of ETS-fusions in our material might be slightly underestimated because of shortage of tumor cells in single biopsies. In the present study, the tumor samples were obtained from patients with more advanced prostate cancer compared with the study reported by Tomlins *et al* (5) who mainly used specimens from radical prostatectomies usually representing early forms of prostate cancer. In accordance with the hypothesis that the fusion is an early event in prostate cancer development that preferentially occurs in low-grade tumors, the number of fusion-positive tumors was lower in the present set of tumors compared to previous studies. However, the true incidence of the various ETS-gene fusions in prostate cancer is still unknown and has to be determined in large, unselected series of histologically confirmed localized and metastatic prostate cancers.

The reason why *ERG*-fusions are much more common in prostate cancer than other ETS-fusions is unknown. The frequency of variant gene fusions may depend on a number of factors, including the transcriptional orientation and the

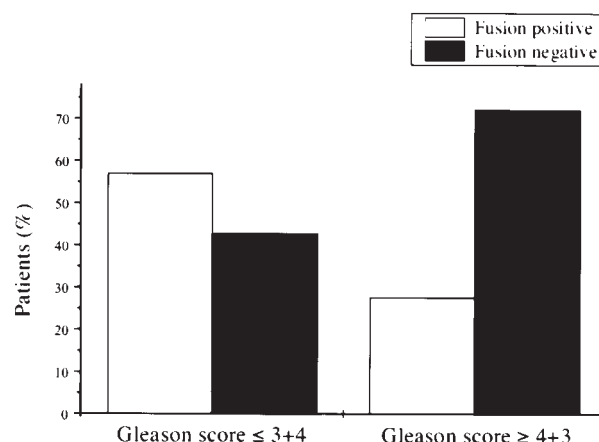


Figure 3. Gleason score in relation to fusion gene status in 50 prostate cancers.

exon-intron organization of the partner genes as well as the size of the introns in which the breakpoints occur (12). Also, factors such as the normal cell type-specific, higher-order spatial organization of chromatin and genes in interphase cells and the nucleotide sequences at the breakpoint junctions may influence the likelihood by which a certain variant gene fusion is generated (12,13). According to publicly available sequence databases, *TMPRSS2* has the same transcriptional orientation as *ERG* and is located only ~3 megabases centromeric to *ERG* within 21q22.3. A fusion between *TMPRSS2* and *ERG* can thus be generated by a simple, cytogenetically cryptic deletion of a small DNA segment containing the 3'-part of *TMPRSS2* and the 5'-part of *ERG* (14), whereas a fusion between for example *TMPRSS2* and *ETVI* requires multiple rearrangements due to the transcriptional orientation of the two genes on different chromosomes. In Ewing's sarcoma, which typically are characterized by fusions of the *EWSR1* gene to members of the ETS-gene family (Fig. 2), *ERG*-fusions are at least 10-times more common than *ETVI*-fusions (15). Since the 5'-partner genes in the prostate- and Ewing-specific ETS-fusions are different, the preference for *ERG* or *ETVI* to participate in a particular gene fusion is likely to be due to inherent functional or structural properties of these genes. Cryptic gene fusions have also been found in several other types of neoplasms, for example in leukemias (16-18), salivary gland mucoepidermoid carcinomas and pleomorphic adenomas (19,20), as well as in sarcomas (21,22). Together with the present results, these and other studies (23) indicate that fusion genes might be much more common in human neoplasms, particularly in epithelial tumors, than previously anticipated.

Recent studies have shown that *ERG* is overexpressed in the majority of prostate cancers and that patients with high *ERG* overexpression (>100-fold or 2-100-fold) in their prostate tumors have significantly longer PSA recurrence-free survival compared to those with lower expression levels (<2-fold) (24). This observation prompted us to examine the clinical and histopathological significance of expression of the *TMPRSS2-ERG* gene fusion in the present set of patients (Table I). There was no statistically significant difference in outcome between fusion-positive and fusion-negative patients with regard to cause-specific survival or progression-free survival (data not shown). The *TMPRSS2-ERG* fusion gene



was found in 21% of patients with a PSA progression-free survival of  $\leq 1$  year and in 41% of patients with a progression-free survival of  $> 1$  year (Table I). Moreover, the fusion was observed in 57% of the tumors with a Gleason score of (3+4 and in 28% of tumors with a Gleason score of  $\geq 4+3$  ( $p=0.099$ ; Table I and Fig. 3). Although there is a clear tendency that fusion-positive tumors are associated with better survival and lower Gleason score than fusion-negative tumors, there were no statistical differences between the groups. This could be related to the fact that the study sample was too small and/or that the fusion bears little or no prognostic information. Additional studies are therefore warranted before firm conclusions can be made as to the clinical significance of a molecular classification of prostate cancers into fusion-positive and fusion-negative tumors.

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