Asian Journal of Andrology (2020) 22, 200–207
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ORIGINAL ARTICLE

Prostate Cancer

Prevalence and clinical application of *TMPRSS2-ERG* fusion in Asian prostate cancer patients: a large-sample study in Chinese people and a systematic review

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Fusion between the transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog (*TMPRSS2-ERG* fusion) is a common genetic alteration in prostate cancer among Western populations and has been suggested as playing a role in tumorigenesis and progression of prostate cancer. However, the prevalence of *TMPRSS2-ERG* fusion differs among different ethnic groups, and contradictory results have been reported in Asian patients. We aim to evaluate the prevalence and significance of *TMPRSS2-ERG* fusion as a molecular subtyping and prognosis indicator of prostate cancer in Asians. We identified the fusion status in 669 samples from prostate biopsy and radical prostatectomy by fluorescence *in situ* hybridization and/or immunohistochemistry in China. We examined the association of *TMPRSS2-ERG* fusion with clinicopathological characteristics and biochemical recurrence by Chi-square test and Kaplan–Meier analysis. Finally, a systematic review was performed to investigate the positive rate of the fusion in Asian prostate cancer patients. McNemar's test was employed to compare the positive rates of *TMPRSS2-ERG* fusion detected using different methods. The positive rates of *TMPRSS2-ERG* fusion were 16% in our samples and 27% in Asian patients. In our samples, 9.4% and 19.3% of cases were recognized as fusion positive by fluorescence *in situ* hybridization and immunohistochemistry, respectively. No significant association between the fusion and clinical parameters was observed. *TMPRSS2-ERG* fusion is not a frequent genomic alteration among Asian prostate cancer patients and has limited significance in clinical practices in China. Besides ethnic difference, detection methods potentially influence the results showing a positive rate of *TMPRSS2-ERG* fusion.

Asian Journal of Andrology (2020) **22**, 200–207; doi: 10.4103/aja.aja_45_19; published online: 14 June 2019

Keywords: Asian; Chinese; prostate cancer; systematic review; *TMPRSS2-ERG*

INTRODUCTION

In the United States, it was estimated that more than 29 000 men would die of prostate cancer (PCa) in 2018.[1] The high incidence and cancer-related death rate of PCa make the disease a serious threat for Western men's health.[2] However, Asians are several times less likely to develop PCa, although PCa morbidity and mortality have been increasing in Asian countries in the last decades.[3,4] These differences may be caused by different lifestyles, environments, medical conditions, and, most importantly, genomic pathogenesis.[5]

Overexpression of v-ets erythroblastosis virus E26 oncogene homolog (*ERG*) mRNA in PCa was first reported by Petrovics and his colleagues in 2005,[6] following which Tomlins and colleagues[7] discovered the mechanism of *ERG* activation to be the fusion between transmembrane protease serine 2 (*TMPRSS2*) and *ERG*. *ERG* expression was promoted by androgen through *TMPRSS2*, which finally resulted in the overexpression of proto-oncoprotein ERG.[8–10] During this period, numerous studies demonstrated that aberrantly expressed *ERG* combined with phosphatase and tensin homolog (*PTEN*) loss or other molecular alterations promoted the oncogenesis and metastasis of PCa both *in vitro* and *in vivo*.[11–16] Moreover, according to data from The Cancer Genome Atlas (TCGA), *TMPRSS2-ERG* fusion is one of the predominant molecular classification factors and promising prognostic markers for localized PCa. *TMPRSS2-ERG* fusion combined with *PCA3* was used in the clinical setting by Tomlins *et al.*[17] to save patients with elevated prostate-specific antigen (PSA) levels referred for biopsy, which potentially decreased the side effects of biopsy and the anxiety associated with waiting for the diagnosis.

However, a series of studies have demonstrated that *TMPRSS2-ERG* fusion has a strong correlation with ethnicity, and the positive rates of *TMPRSS2-ERG* fusion differ among different ethnic and geographical groups, at a wide range of 7%–83%.[18–20] Although more than half of PCa patients in North America and Europe harbor the *TMPRSS2-ERG* fusion,[21,22] it is still controversial whether it is a common gene fusion type in Asian patients.[18,23] A rising number of studies have focused on

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Received: 16 October 2018; Accepted: 27 March 2019

expounding the interaction of *TMPRSS2-ERG* fusion and PCa in Asia, and with conflicting results. In 2010, Sun and colleagues[24] examined *TMPRSS2-ERG* fusion in 50 Chinese PCa samples by fluorescence *in situ* hybridization (FISH) and found 39 (78.0%) positive cases. However, another Chinese researcher found that *TMPRSS2-ERG* fusion was detected by the same method in only 7 (7.5%) of 93 Chinese patients.[25]

There is evidence that unstandardized detection methods, including FISH, immunohistochemistry (IHC), polymerase chain reaction (PCR), and some other high-throughput methods, may produce different results in detecting *TMPRSS2-ERG* fusion.[23,26] The disparities in the positive rates of *TMPRSS2-ERG* raise the question of whether there is equal applicability of this genomic alteration in Asian patients.

In the present study, we aimed to evaluate the positive rate of *TMPRSS2-ERG* fusion in Asian patients by experiment and a systematic review and to assess its clinical significance as a cancer biomarker in Chinese people. We also made efforts to investigate the factors which could influence the measured positive rate of *TMPRSS2-ERG* fusion.

PATIENTS AND METHODS

Patients and prostate specimens

Paraffin-embedded tissue blocks of 729 consecutive PCa patients who underwent radical prostatectomy or prostate biopsy in Shanghai Changhai Hospital (Shanghai, China), between January 2010 and July 2018, were retrieved from the Hospital's Department of Pathology. Two independent pathologists reviewed corresponding hematoxylin and eosin (H&E)-stained slides of each block (6–15 blocks per patient) to confirm pathological diagnosis, and 669 eligible blocks were selected. For each patient receiving biopsy in the hospital, 12 cores were obtained and the core with the greatest tumor volume was chosen for the experiment. Age at diagnosis, body mass index (BMI), preoperative PSA, Gleason pattern, emission computed tomography (ECT) diagnosis, clinical tumor stage, and perineural and lymphovascular invasion status were retrieved from medical records, and patients' follow-up was conducted in accordance with the Chinese Guidelines for the Diagnosis and Treatment of Urological Diseases.[27] Biochemical recurrence (BCR)-free survival is defined by a PSA level ≥0.2 ng ml^{-1} in two successive follow-ups after surgery. Informed consent was obtained from the patients before surgery, and all procedures performed in this study involving human participants were approved by the Institutional Review Board of Shanghai Changhai Hospital.

Fluorescence in situ hybridization and immunohistochemistry

We detected DNA fusion by *ERG* break-apart FISH assay, which was demonstrated to be a reliable technique to detect the fusion between two neighboring genes.[10,28] Bacterial artificial chromosome (BAC) clones and FISH assay kits (F01015) were obtained from GP Medical Technologies (Beijing, China). Fluorescein (green)-labeled RP11-24A11 and tetramethylrhodamine (red)-labeled RP11137J13 were provided in the FISH assay kit, which spanned the centromeric and telomeric regions of the *ERG*, respectively. The experiment was performed following the manufacturer's instructions. Briefly, 4-μm sections were deparaffinized and dehydrated followed by Proteinase K digestion (provided in the FISH assay kit). After washing and fixation, the sections were dehydrated and dried. Denaturation was under 85°C for 10 min and hybridization was under 42°C overnight.

Fluorescent images were captured by a ×100 oil lens (Olympus BX51, Tokyo, Japan). A normal cell exhibits a pair of orange signals in nucleus while cells with gene fusion show separated red and green coloring or lack one of these colors. For each case, we counted at least 100 nuclei, and fusion was recorded when there were more than 10% of nuclei exhibited abnormal signals.

Immunohistochemistry

IHC analysis of ERG expression was performed on 4-μm sections using an UltraSensitive TMS-P kit (KIT-9710, MaiXin Biotechnology, Fujian, China). The tissue sections were dewaxed, followed by gradual dehydration. Then, heat-induced antigen retrieval was processed in 0.01 mol l^{-1} citrate buffer in a microwave for 15 min. Primary antibody incubation for ERG (1/200, ab92513, Abcam, Cambridge, UK) was conducted at 4°C overnight and secondary antibody was included in the kit. DAB staining was performed with a DAB staining kit (DAB-2031, MaiXin Biotechnology) as per the manufacturer's instructions. Slides were scanned using a Nano Zoomer S60 (Hamamatsu Photonics, Iwata City, Japan), and ERG expression status was recorded as negative (no staining or stained area <10%) or positive (weak or strong staining).

Statistical analyses

A Chi-square test was employed for comparing the association between the fusion status and clinical characteristics. BCR-free survival rate was calculated using Kaplan–Meier analysis and a log-rank test. McNemar's test was used to compare the difference between positive rates of gene fusion evaluated by FISH and IHC. Statistical analysis were analyzed using SPSS (IBM SPSS Statistics for Windows, version 19.0, IBM Corp., Armonk, NY, USA), and graphs were drawn using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). Statistics were considered statistically significant when two-sided $P < 0.05$.

Publication search

The systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Literature was searched for in the PubMed and Embase databases on December 20, 2017, with no restrictions on publication year. The following search terms were used: "TMPRSS2," "ERG" OR "ETS related gene," AND "Prostate cancer," and both the adjective and noun forms of the name of each Asian country or region. Only abstracts or articles in English were included. Two authors (RC and DPK) independently reviewed the articles, and fusion-related information was extracted.

Meta-analysis

Heterogeneity among studies was measured using the Cochrane Q statistic ($P > 0.05$ for homogeneity) and the I^2 statistic. I^2 is calculated using the formula: $I^2 = (Q - df)/Q$, in which df means degree of freedom. $I^2 < 40\%$ was considered to be that no important heterogeneity existed and $I^2 > 75\%$ was considered to be that heterogeneity existed.[29] The fixed effects model and random effects meta-analysis were applied as being relevant. All statistical analyses for the meta-analysis were performed using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria) with the Meta libraries.

RESULTS

TMPRSS2-ERG fusion and its clinical association

Table 1 summarizes the clinicopathological characteristics of patients included in the study. In the study, the mean follow-up time of patients was 26.6 (range: 0–96.0) months, and the median was 22.0 months. A total of 669 patients with 179 biopsy samples and 490 prostatectomy samples were evaluated. The mean age of all patients was 67.8 (range: 43–88) years, and biochemical recurrence was observed in 7.5% (22/333) patients during follow-up. Among the 669 samples, 110 (16.4%) showed the *TMPRSS2-ERG* fusion. Samples from

Table 1: Clinicopathological characteristics of 669 investigated patients

Characteristics	*Biopsy (n=179)*	*Prostatectomy (n=490)*	*All*
Age (year), mean±s.d.	70.0±8.3	67.0±7.1	67.8±7.6
BMI (kg m^{-2}), mean±s.d.	24.1±2.9	24.6±3.1	24.5±3.1
PSA level (ng ml^{-1}), *n* (%)			
<4	38 (21.2)	62 (12.7)	100 (15.0)
4–10	37 (20.7)	109 (22.2)	146 (21.8)
>10	102 (57.0)	319 (65.1)	421 (62.9)
Unknown	2 (1.1)	0 (0)	2 (0.3)
Major Gleason score, *n* (%)			
3	63 (35.2)	219 (44.7)	282 (42.2)
4	79 (44.1)	218 (44.5)	279 (41.7)
5	37 (20.7)	53 (10.8)	90 (13.4)
Sum of Gleason score, *n* (%)			
6	21 (11.7)	91 (18.6)	112 (16.7)
3+4	41 (23.9)	119 (24.3)	160 (23.9)
4+3	22 (22.9)	68 (13.9)	90 (13.4)
>7	95 (53.1)	212 (43.3)	307 (45.9)
Clinical tumor stage, *n* (%)			
T0/T1	28 (15.6)	112 (22.9)	140 (20.9)
T2	65 (36.3)	275 (56.1)	340 (50.8)
T3	36 (20.1)	81 (16.5)	117 (17.5)
T4	28 (15.6)	2 (0.4)	30 (4.5)
Unknown	22 (12.3)	20 (4.1)	42 (6.3)
Aberrant bone scan, *n* (%)	54 (30.2)	70 (14.3)	124 (18.5)
Perineural invasion[a], *n* (%)	NA	241 (49.7)	NA
Lymphovascular invasion[a], *n* (%)	NA	41 (15.1)	NA
Biochemical recurrence[a], *n* (%)	NA	25 (7.5)	NA

[a]Missing data in prostatectomy, 5 for perneural invasion; 218 for lymphovascular invasion; 157 for biochemical recurrence. s.d.: standard deviation; BMI: body mass index; PSA: prostate-specific antigen; NA: not available

prostatectomy had a higher fusion rate than those from biopsy tissue (17.8% *vs* 12.9%) though the data were not statistically significant. Similarly, patients with higher BMI seemed more likely to harbor *TMPRSS2-ERG* fusion. However, a Chi-square test suggested that none of the investigated clinicopathological characteristics were associated with *TMPRSS2-ERG* fusion. **Table 2** shows related details. In addition, Kaplan–Meier survival analysis showed no difference in BCR rates between the fusion-positive and fusion-negative groups (**Figure 1**).

TMPRSS2-ERG fusion rates by different detection methods

Samples were randomly assigned into subgroups, in which they were detected by different methods. We evaluated 73 biopsy samples and 194 prostatectomy samples by FISH, and 84 biopsy samples and 196 prostatectomy samples by IHC. In addition, 22 biopsy samples and 100 prostatectomy samples were simultaneously assessed by FISH and IHC. *TMPRSS2-ERG* fusion was considered positive as long as either of FISH and IHC, or both, detected fusion signals. **Supplementary Table 1** and **2** show the number of different sample types and positive rates of samples evaluated by FISH, IHC, or both. Of the 267 samples detected by FISH, 25 (9.4%) were identified as fusion positive while 54/280 (19.3%) fusion was found by IHC. It was surprising that samples were more likely to be defined as fusion positive by IHC than by FISH ($P < 0.001$). There were 17 cases recognized as fusion positive by IHC and contradictorily negative by FISH. However, no case detecting positive signals by FISH was recognized as fusion negative by IHC. **Figure 2** shows representative images of FISH and IHC.

Literature search and study selection

Using the search strategy described above, we identified a list of 184 and 295 studies from PubMed and Embase, respectively. Abstracts of 243 studies were carefully reviewed and studies carried out by Asian authors but investigating non-Asian populations, or studies with experiments performed only in PCa cell lines, were excluded. The abstracts and full articles of the remaining studies were then screened. Consequently, 45 studies with 5371 cases were included in the meta-analysis. **Figure 3** shows a flowchart of the literature research.

Overall pooled results of TMPRSS2-ERG fusion in Asian patients

The pooled results indicated that the positive rate of *TMPRSS2-ERG* fusion was 27% (95% confidence interval [CI]: 24%–32%) in all included studies. Among the 49 selected records, 18 reported a positive rate of 20% or lower and 17 reported a positive rate of 30% or higher. The highest positive rate (78%) was observed in the study by Sun *et al.*[24] and the lowest detection rate (0) was observed in the study by Furusato *et al*.[30] **Figure 4** shows a forest plot for the 45 studies.

Positive rate of TMPRSS2-ERG fusion by subgroup analysis

Studies were divided into groups to compare the differences between different populations, sample types, detection methods, and study sample size. Most studies were from China ($n = 17$), South Korea ($n = 11$), or Japan ($n = 8$), and patients from these three countries accounted for 86.8% of all patients. The positive rate of *TMPRSS2-ERG* fusion in the Japanese population was 21% (95% CI: 17%–25%), which was relatively lower than 25% (95% CI: 17%–34%) in Chinese and 26% (95% CI: 20%–32%) in South Koreans. However, Indian and Turkish populations were reported to have higher fusion rates (52% and 46%, respectively). After excluded Aryan and Caucasian population, the positive rate of the fusion in Asians was 24% (95% CI: 20%–29%). **Supplementary Table 3** lists the prevalence of the fusion in different countries examined. It is noteworthy that fusion

Table 2: Association of transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog fusion status with clinical parameters among 669 prostate cancer samples

Parameters	*Fusion*		*Positive rate (%)*	*P*
	Negative (n)	*Positive (n)*		
PCa samples	559	110	16.4	
Age (year)				0.57
≤65	187	40	17.6	
>65	370	70	15.9	
BMI (kg m^{-2})				0.11
<19	12	2	14.3	
19–27	447	82	15.5	
>27	92	25	21.4	
Sample type				0.13
Biopsy	156	23	12.8	
Prostatectomy	403	87	17.8	
PSA level (ng ml^{-1})				0.31
<4	87	13	13.0	
4–10	117	29	19.9	
>10	353	68	16.2	
Unknown	2	0	0	
Major Gleason score				0.44
3	230	52	18.4	
4	254	43	14.5	
5	75	15	16.7	
Sum of Gleason score				0.93
6	94	18	16.1	
3+4	129	31	19.4	
4+3	76	14	15.6	
>7	260	47	15.3	
Clinical tumor stage				0.79
T0/T1	115	25	17.9	
T2	287	53	15.6	
T3	100	17	14.5	
T4	24	6	20.0	
Unknown	33	9	21.4	
Perineural invasion				0.67
Positive	197	44	18.3	
Negative	203	41	16.8	
Unknown	159	25	13.6	
Lymphovascular invasion				0.55
Positive	38	7	15.6	
Negative	183	44	19.4	
Unknown	182	36	16.5	
Biochemical recurrence				0.70
Positive	21	4	16.0	
Negative	257	51	16.6	
Lost	125	32	20.4	

BMI: body mass index; PSA: prostate-specific antigen; *TMPRSS2-ERG*: transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog; PCa: prostate cancer

detected in samples from prostate biopsy (34%; 95% CI: 24%–47%) was higher than that in samples from radical prostatectomy (24%; 95% CI: 20%–29%). Samples assessed by PCR showed higher fusion rate (40%; 95% CI: 26%–55%) than samples assessed by IHC (26%; 95% CI: 22%–31%) and FISH (25%; 95% CI: 18%–35%).

The positive rates of fusion in the smaller sample-sized ($n \leq 30$) group and larger sample-sized ($n > 30$) group was 33% (95% CI: 17%–56%) and 27% (95% CI: 23%–31%), respectively. This implied that a sampling error in some studies may have caused higher prevalence of the fusion.

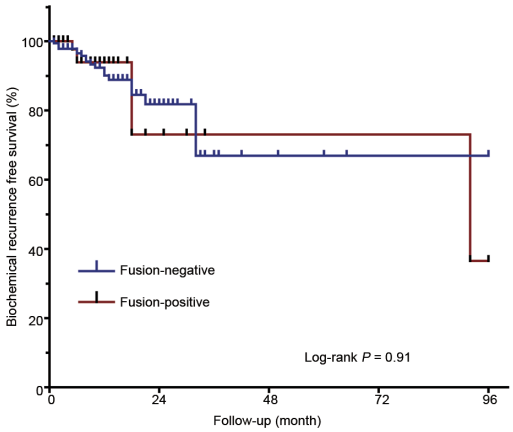

Figure 1: BCR-free survival rates of 490 patients receiving radical prostatectomy. Kaplan–Meier analysis was performed and no significant difference was observed in BCR-free survival rates between the fusion-positive and fusion-negative groups (log-rank $P = 0.91$). BCR: biochemical recurrence.

DISCUSSION

Gene fusions have been recognized as frequent events in diseases including cancer since Peter Nowell and David Hungerford reported BCR-ABL1 fusion in chronic myeloid leukemia (MCL) in the 1960s.[31,32] In PCa, *TMPRSS2-ERG* fusion is one of the most well-known genomic alterations and a large number of studies have been carried out to investigate the function and application of this fusion as an oncogenic factor and a diagnostic or prognostic biomarker.[33,34] Nevertheless, these reports were mostly in the Western populations, and the value of *TMPRSS2-ERG* fusion in Asian patients is quite unclear.

Several studies have reported that Eastern Asian patients are two to five times less likely to harbor the fusion.[35–38] We confirm that 110 (16.4%) of the 669 Chinese PCa patients harbor *TMPRSS2-ERG* fusion, which coincides with our previous report that ERG protein was overexpressed in 14.9% (26/174) of cases in tissue arrays in our hospital.[39] Furthermore, the meta-analysis indicates that 27% (95% CI: 23%–31%) of Asian patients are fusion positive, which is approximately half the rate in the Western populations and is consistent with previous multiracial studies.[10,19,40] Exceptions were Indian (Aryan descent) and Turkish (Caucasian descent), of which the fusion rates were 52% (95% CI: 43%–60%) and 46%, respectively, in our meta-analysis. Rawal *et al.*[41] conducted the first investigation into the positivity of *TMPRSS2-ERG* fusion in an Indian population in 30 evaluable samples, of which they found 8 (27%) fusion-positive cases. They concluded that the positivity of *TMPRSS2-ERG* fusion in Indian patients was relatively lower, though this was not supported by other following studies. One year later, Jain *et al.*[42] reported a fusion positive rate of 64% in Indian patients. In 2015, Suryavanshi *et al.*[43] and Ateeq *et al.*[44] detected the fusion in 51 of 100 and 46 of 94 samples, respectively. However, this was strong evidence of racial difference in *TMPRSS2-ERG* fusion.

The clinical utility of *TMPRSS2-ERG* fusion as a diagnostic and prognostic biomarker of PCa remained under debate both in the Western and Eastern populations. *TMPRSS2-ERG* fusion has been demonstrated as associated with PSA level, Gleason grade, tumor stage, metastasis, and BCR or tumor-specific death in some studies.[10,23,45–47] In a multicenter study involving 1312 patients, Tomlins and colleagues[17]

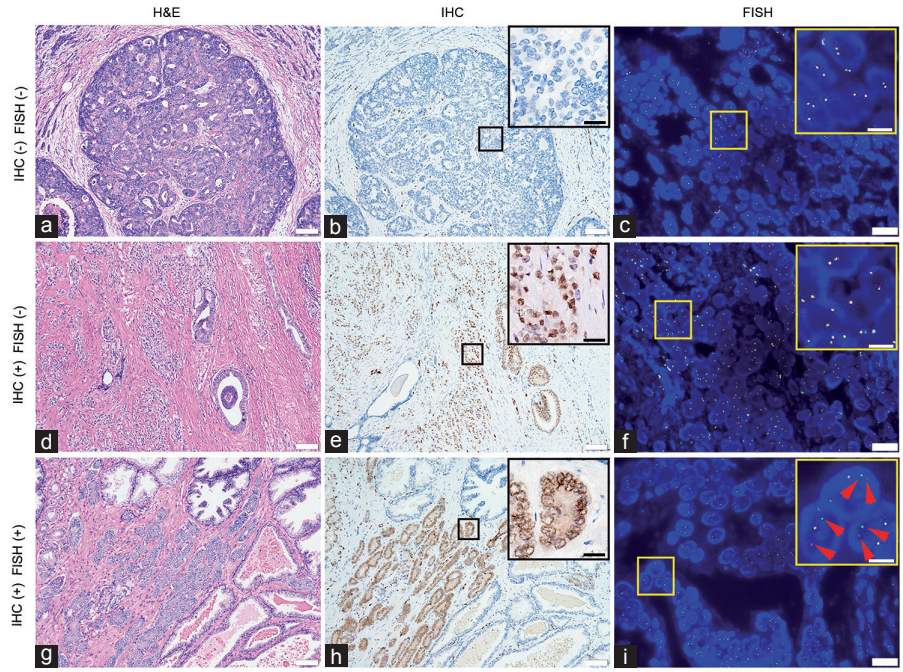

Figure 2: H&E stains, IHC, and FISH images of three cases showing different fusion status. Small boxes indicate areas shown at higher magnification in the larger box. (**a**) H&E stain of a fusion-negative case of prostate cancer with cribriform glands (Gleason grade 4). (**b**) IHC shows positive signals in some of the blood vessel endothelium, but no ERG expression in cancerous prostate glands. (**c**) In FISH images, there was no separation of red and green signals. (**d**) H&E stain of a case recognized as fusion positive by IHC, but negative by FISH. (**e**) Strong signals of ERG expression can be seen in the IHC image, but (**f**) almost all the cells exhibit normal signals in the FISH image. (**g**) H&E stain of one case of prostate cancer (Gleason grade 3) evaluated as fusion positive by IHC and FISH. (**h**) IHC shows ERG expression in cancerous prostate glands. (**i**) In large portion of cell nuclei, one yellow, one red (red arrows), and one green signal (red arrows) indicate *TMPRSS2-ERG* fusion through translocation. Scale bars = 100 μm in **a**, **b**, **d**, **e**, **g** and **h**; 30 μm in up-right image of **b**, **e** and **h**; 20 μm in **c**, **f**, and **i**; 7.5 μm in up-right image of **c**, **f**, and **i**. H&E: hematoxylin and eosin; IHC: immunohistochemistry; FISH: fluorescence *in situ* hybridization; TMPRSS2: transmembrane protease serine 2; ERG: v-ets erythroblastosis virus E26 oncogene homolog.

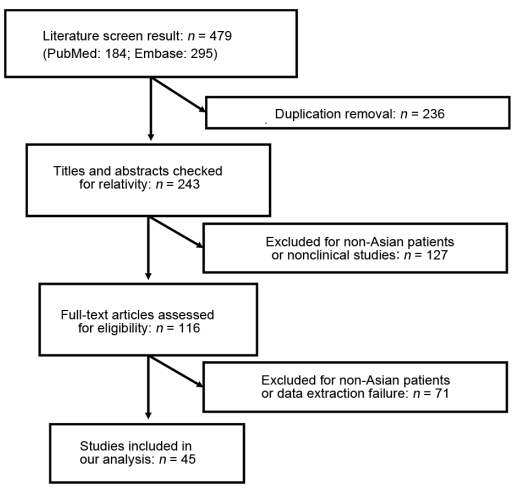

Figure 3: Flowchart of literature screening with inclusion and exclusion criteria.

put forward that urine *TMPRSS2-ERG* fusion was associated with tumor size, high Gleason score, and upgrading of Gleason score at prostatectomy. However, two prospective studies from Smith and colleagues demonstrated that *TMPRSS2-ERG* fusion in prostatic secretion could predict neither early BCR among patients receiving prostatectomy[48] nor Gleason upstaging among patients receiving active surveillance[49] in the US population. It was also observed in Asia that no consensus on the clinical significance of *TMPRSS2-ERG* fusion was reached. Kim and colleagues reported better BCR-free survival rates among fusion-positive patients[50] while Lee found the fusion in Korean patients had no relation with BCR but strong correlation with lower Gleason grade.[38] In our study, no significant association between *TMPRSS2-ERG* fusion and clinical parameters mentioned above was confirmed.

We observed that the positive rate of *TMPRSS2-ERG* fusion was lower in biopsies (12.9%) than in prostatectomy specimens (17.8%) in our hospital, but with no statistical significance (P = 0.13). This result is supported by Mosquera and other researchers who found that specimens from radical prostatectomy and biopsy had the equal positive rate of *TMPRSS2-ERG* fusion.[10,21,51] Controversially, our meta-analysis reported a higher positive rate in biopsy specimens (35%) than in radical prostatectomy specimens (24%). One reason may be the disparity of the sample distribution; Indian patients accounted for a larger proportion in the biopsy group (26%) than in the prostatectomy group (2%).

We compared FISH with IHC in detecting *TMPRSS2-ERG* fusion in our samples and found that IHC produced a higher positive rate. In other studies, researchers found that *TMPRSS2-ERG* fusion was detected in 30% of patients by FISH in the UK[46] and the US,[52] while van Leenders *et al.* [53] reported that the positive rate of the fusion among US patients was 61% using a specific antibody for fusion generated ERG. Cross-reactivity of antibodies may be responsible for the higher sensitivity, and compared with FISH, which provides direct evidence of gene fusion, IHC is more likely to produce false-positive results. Thus, the actual positive rate of the fusion could be lower than we detected. In our meta-analysis, PCR detected a higher positive rate of fusion

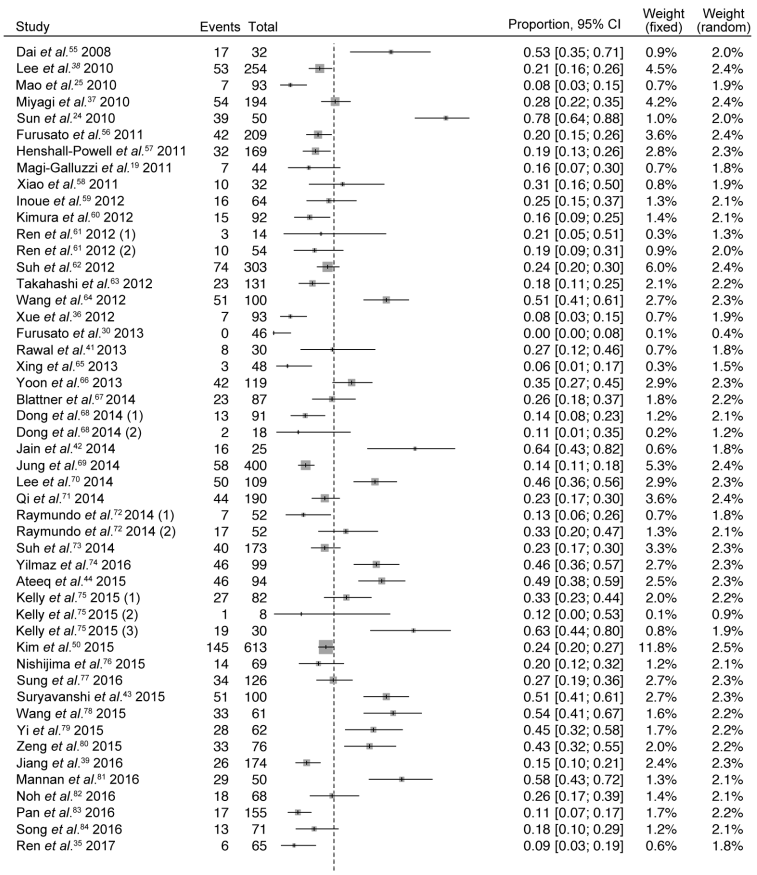

Figure 4: Forest plot of positive rate of *TMPRSS2-ERG* fusion for 45 included studies. A random effects model is used and the positive rate of *TMPRSS2-ERG* fusion in Asian PCa patients is 27%. Data were individually analyzed when two or more groups were included in one research and we present these subgroups as (1), (2), (3). CI: confidence interval; *TMPRSS2-ERG*: transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog; PCa: prostate cancer.

than FISH or IHC. However, Hagen and other researchers found that RT-PCR was as reliable as FISH in detecting the fusion.[26]

To the best of our knowledge, this study has the largest sample size in Asia reporting the *TMPRSS2-ERG* fusion rate. In addition, a positive rate of fusion was calculated from the latest studies by a meta-analysis among Asian population. However, several limitations should be taken into account in this study. It was a single-center study and most patients were from Eastern China; the long-term prognostic value of *TMPRSS2-ERG* fusion in our study needs to be updated in follow-up work. The antibody used in the IHC detects both ETS transcription factor ERG (EGR) and Fli-1 proto-oncogene (FLI-1), resulting in the increase of false-positive cases. However, Paulo and colleagues found that FLI-1 protein was expressed in only 1 of 200 PCa patients, indicating that FLI-1 could contribute a limited false-positive rate in detecting ERG by IHC in PCa samples.[54]

CONCLUSION

The positive rate of *TMPRSS2-ERG* fusion is much lower in PCa among a Chinese population, and this gene aberration does not correlate with PSA level, Gleason grade, clinical tumor stage, bone metastasis, perineural invasion, lymphovascular invasion, or BCR of patients in the present study. The systematic review confirmed that the positive rate of *TMPRSS2-ERG* fusion is much lower in Asian PCa patients. Based on our data, we believe that some of the results from Asian studies have been possibly affected by their detection methods and sample size. We assert that *TMPRSS2-ERG* fusion may be less effective as a diagnostic and prognostic biomarker in Asians because of its low prevalence and insignificant correlation with clinical parameters. Furthermore, it appears important and urgent to find suitable molecular and genomic biomarkers in Asian PCa patients.

AUTHOR CONTRIBUTIONS

XG and YHS contributed to conceptualization and project administration. DPK and GAX performed data curation. WZ and FBW were in charge of formal analysis. RC and YHS contributed to funding acquisition. DPK contributed to investigation and visualization. DPK, CLZ, and NT contributed to methodology. YHS collected the resources. DPK and RC contributed to software, validation and writing the original draft. DPK, RC, and CLZ wrote, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This research was funded by the National Nature Science Foundation Youth Project (Grant No. 81702514), the National Natural Science Foundation of China (Grant No. 81430058), and the Clinical Research Project of Shanghai Municipal Commission of Health and Family Planning (Grant No. 20184Y0130).

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog fusion detected in different sample types by fluorescence *in situ* hybridization and/or immunohistochemistry

Methods	*Fusion positive/total (%)*		
	Biopsy	*Prostatectomy*	*Total*
FISH	9/73 (12.3)	16/194 (8.2)	25/267 (9.4)
IHC	9/84 (10.7)	45/196 (23.0)	54/280 (19.3)
FISH and IHC	5/22 (22.7)	26/100 (26.0)	31/122 (25.4)

FISH: fluorescence *in situ* hybridization; IHC: immunohistochemistry

Supplementary Table 2: Comparison of number of fusion positive cases among 122 samples detected by fluorescence *in situ* hybridization and immunohistochemistry simultaneously

FISH	*IHC*		*Total*	*P*
	Fusion negative	*Fusion positive*		
Fusion negative	91	0	92	<0.001
Fusion positive	17	14	30	
Total	108	14	122	

FISH: fluorescence *in situ* hybridization; IHC: immunohistochemistry

Supplementary Table 3: Prevalence of transmembrane protease serine 2 and v-ets erythroblastosis virus E26 oncogene homolog fusion stratified by examined countries in Asia

Country	*Pooled positive rate (%)*	*95% CI*	*Patient (n)*
Korea	25	21–30	2323
China	25	17–34	1490
Japan	21	17–25	849
India	52	43–60	329
Philippines	23	-	104
Turkey	46	-	99
Malesia	13	-	8

CI: confidence interval