Original Article

Status of *TMPRSS2–ERG* fusion in prostate cancer patients from India: correlation with clinico-pathological details and *TMPRSS2* Met160Val polymorphism

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ABSTRACT

Background: Prostate cancer (PCa) shows considerable clinical heterogeneity that has been primarily attributed to variable molecular alterations. *TMPRSS2–ERG* fusion is one such molecular subtype that has been associated with predominantly poor prognosis. More recently, a single nucleotide polymorphism (SNP) in the *TMPRSS2* gene rs12329760 C>T (Met160Val) has been shown to positively correlate with the fusion status and also to be associated with increased risk for PCa. The aim of the present study is to determine the frequency of *TMPRSS2–ERG* fusion and association of rs12329760 in Indian PCa patients with fusion status.
Methods: *TMPRSS2–ERG* fusion by fluorescence *in situ* hybridization was determined in 102 of 150 PCa biopsy-proven cases. Genotyping for rs12329760 was performed on the entire cohort of 150 cases by Sanger sequencing.
Results: *TMPRSS2–ERG* fusion was seen in 27 of 102 (26%) cases. Fusion-positive patterns in this study showed fusion by translocation in nine of 27 cases (33.5%), by deletion in six of 27 (22%) cases, and by insertion in 12 of 27 cases (44.5%). No association of the fusion status with Gleason Score, pattern, or perineural invasion was seen. The *TMPRSS2* SNP rs12329760 'T' allele was prevalent with a frequency of 0.27 in the PCa patients. The SNP was significantly associated with fusion [odds ratio (OR) = 2.176, 95% confidence interval (CI) = 1.012–4.684, $P = 0.04$], more specifically fusion by deletion ($P = 0.04$).
Conclusion: The results provided here determine the frequency of *TMPRSS2–ERG* fusions (26%) in a fairly large cohort of Indian PCa cases and also the association of rs12329760 SNP with *TMPRSS2–ERG* fusion. No association with other clinico-pathological features was observed. Future studies with clinical outcomes are warranted in this population.

1. Introduction

Prostate cancer (PCa) is a heterogeneous disease, which ranges from indolent to lethal behavior.[1] The current diagnostic modalities i.e., serum prostate-specific antigen (PSA), digital rectal examination, and histopathologic examination often lead to both over-diagnosis and overtreatment.[2]

In 2005, a novel set of fusion genes were described in nearly half of the PCa cases involving the 5′-untranslated region of *TMPRSS2* (21q22) and the codifying region of some transcription factors such as ERG (21q22), ETV1 (7p21),[3] and later ETV4 (17q21), defined as a third molecular subtype.[4] This generated considerable interest to evaluate *TMPRSS2–ETS* fusions with PCa risk and as a potential diagnostic and prognostic indicator.

TMPRSS2 is a prostate-specific, androgen-responsive, trans-membrane serine protease. ETS family members are oncogenic transcription factors; therefore, the fusion of these genes leads to the production of ETS transcription factors under the control of the androgen-sensitive promoter elements of TMPRSS.[4]

The most common TMPRSS fusions is with ERG (ETS-related gene), resulting in the *TMPRSS2–ERG* fusion, which has been

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identified in approximately from 23% to 50% of PCa cases in different cohorts.[5]

The main mechanisms by which the *TMPRSS2−ERG* rearrangement occurs are either by a ~3-Mb interstitial deletion on a single copy of chromosome 21 or by a chromosomal translocation. The high prevalence of *TMPRSS2−ERG* fusions suggests that this region is a hot spot for chromosomal rearrangements in PCa. These fusions can be detected by fluorescence *in situ* hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), or immunohistochemistry (IHC). But, a meta-analysis has implied that some if not all methods may misclassify *TMPRSS2−ERG* fusion status to some degree.[5]

Correlation of the fusion status with clinical outcomes for PCa has yielded mixed information. Fusion status has been linked with favorable prognosis in few studies,[6,7] but with poor outcomes in others.[8] Fusion-positive status has also been associated with recurrence and aggressiveness of the disease in a few studies.[9−11] Interestingly, it has also been indicated that fusion formed via deletion, rather than translocation, be associated with aggressive disease[12] and also with higher tumor stage and lymph node involvement in PCa patients.[13] The majority of findings however indicate that the presence of *TMPRSS2−ERG* fusion gene expression in PCa patients is associated with poor clinical prognosis.[7]

Correlation with other parameters such as stage, grade, and Gleason score did not yield association in a few studies.[8,14,15] In contrast, *TMPRSS2−ERG* fusions were found to be associated with high levels of PSA, advanced stage, and high Gleason scores by Rostad et al[16] and another group.[17]

In addition, a single nucleotide polymorphism (SNP) in *TMPRSS2* rs12329760 C>T (Met160Val) has been found to be associated positively with *TMPRSS2−ERG* fusion by translocation ($P = 0.05$) and multiple copies of the gene fusion ($P = 0.03$).[8] The Met160Val amino acid is highly conserved across mammals; this SNP is present in an exonic splicing enhancer srp40 site, and the presence of the variant allele would result in an increased chance of exon skipping or protein malformation due to potential disruption of the exonic splicing enhancer site.[8]

The *TMPRSS2* Met160Val has shown to be a genetic risk factor for sporadic PCa in a Japanese population.[18] The variant was indicated to be informative of time to PCa diagnosis for a subset of high risk Caucasian men undergoing regular PCa screening.[19] Importantly, the frequency of this SNP differs significantly with race and ethnicity.[20] In this context, it becomes imperative to evaluate genetic variants in different populations.

The aim of the present study is to determine the frequency of *TMPRSS2−ERG* fusion gene and *TMPRSS2* SNP rs12329760 and correlation with the fusion status.

2. Materials and methods

2.1. Subject group

A total of 150 cases with the histology of PCa were included in the study. The demographic data, histology, and type of biopsy were analyzed. For the histopathological examination, the formalin-fixed paraffin-embedded (FFPE) tissue blocks were processed, and slides were cut at 5-μm thickness and stained with routine hematoxylin−eosin stain. The cases were reviewed by two surgical pathologists for varying histological features. The presence of PCa cells was verified by pathological examination of a section stained by hematoxylin−eosin stain adjacent to the slide used for FISH analysis.

This study is in accordance with the Declaration of Helsinki. The study was approved by SRL Ethics Committee.

FISH: FISH was performed using Kreatech diagnostics (Leica Biosystems, Nussloch, Germany) reagents according to

manufacturers' protocol, and the signals were observed on a fluorescence microscope (BX 60, Olympus, Germany). The *TMPRSS2−ERG* rearrangement probe is optimized to detect the deletion between *TMPRSS2* and *ERG* at 21q22 associated with the *TMPRSS2−ERG* fusion in a triple-color deletion assay. It also detects translocations involving the *TMPRSS2* region such as ETV1 t(7:21) or ETV4 t(17:21). Loss of the proximal *TMPRSS2* region is observed as loss of a green signal leaving a red/blue signal at 21q22; translocation at 21q22 results in a single red and green/blue signal pattern at the derivative chromosomes when only *TMPRSS2* is involved and observed as a single blue and red/green signal pattern at the derivative chromosomes when only ERG is involved. Only red and green/blue signals that are more than one signal diameter apart from each other are counted as a break. Single color fusion (RGB) signals will identify the normal chromosomes 21.

A total of 50 epithelial nuclei per case were evaluated across the three cores, and to be classed positive for the *TMPRSS2−ERG* fusion, evidence needed to be present in at least 10−20% of the cells. Evaluation of the FISH results from each case was independently performed by two operators.

2.2. DNA extraction

Genomic DNA was extracted from the collected FFPE tissue using QIAGEN (Hilden, Germany) DNA extraction kit.

2.3. Genotyping

Genotyping was performed for the rs12329760 by direct sequencing of the products obtained after amplification using the primers described below. Sequencing was done bidirectional on the Automated ABI prism 3100 Avant Genetic Analyzer (Applied Biosystems Inc., Foster city, California) using ABI prism BigDye terminator kit (version 3.1).

rs12329760 F: 5′ TCTGCTGTCTGTTACTGTCACT 3′
rs12329760 R: 5′ ACTCATGGATAATCCTCCCTC 3′

2.4. Statistical analysis

Demographic and clinical characteristics were compared between all samples (including PCa cases whose tumor could not be scored) and those who were positive or negative for the *TMPRSS2−ERG* fusion. Associations between genotyping data and *TMPRSS2−ERG* fusion status were examined using Fisher's exact test. For genotyping, allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Any deviation of the genotype frequencies from the Hardy−Weinberg equilibrium was assessed by Fischer's exact test. Chi-square tests were used for comparison of binary variables across groups. Routine statistical analysis was carried out with the SPSS v 15 software (SPSS Inc., Chicago, IL). SNPStats online software tool was applied to determine the association of genotypes with the *TMPRSS2−ERG* fusion. The association was tested using logistic regression with a genotypic genetic model. All reported *P* values were two sided.

3. Results

FFPE blocks of 150 PCa patients were collected along with their clinical reports and history. Genotyping for rs12329760 SNP and FISH analysis to determine the *TMPRSS2−ERG* gene fusions were performed on the same samples. Of the 150 prostate tumors, 102 were scored (68%) and 48 (32%) were not scored because of technical issues (failed hybridization or core drop-off).

The general characteristics of the 150 PCa samples were compared with the fusion-positive and fusion-negative samples, and their statistical relevance was judged using a Chi-square test (Table 1). The Gleason score was seen to be >7 in 59.3% of the fusion-positive samples and in 56.7% of the fusion-negative samples, showing no statistical significance ($P = 0.525$). Similarly, perineural invasion was seen in 62% of the fusion-positive and 59.67% of the fusion-negative samples ($P = 0.875$).

The frequency of TMPRSS2−ERG rearrangement variants is indicated in Table 2. Fusion-positive patterns in this study showed fusion by translocation in nine of 27 cases (33.5%), by deletion in six of 27 (22%) cases, and by insertion in 12 of 27 cases (44.5%). No statistically significant differences were seen in terms of Gleason score between fusion-positive and fusion-negative cases. Similarly, no differences were seen on comparison in terms of perineural invasions ($P > 0.05$). The TMPRSS2−ERG fusion status of the samples with its Gleason score data based on their mean primary, secondary, and total scores was also analyzed (Table 3).

The genotypic and allelic frequencies of rs12329760 are as follows: The total cohort had 56% C/C, 33% C/T, and T/T 10.6%. In fusion-positive cases, the highest frequency of the risk allele T/T (18.5%) was seen (Table 4).

Association of rs12329760C>T with fusion status is indicated in Table 5. Significant association with TMPRSS2−ERG fusion was seen with the T allele (CC vs. CT + TT) ($P = 0.04$).

Further to determine if the association of the genotype was with a particular rearrangement, univariate logistic regression was performed analyzing rs1239760 with TMPRSS2−ERG translocation, insertion or interstitial rearrangement (Table 6). Interestingly, significant association of rs1239760 was seen with interstitial deletion with the dominant model ($P = 0.04$).

The rs12329760 SNP genotypic data distribution with respect to the Gleason score values was also determined. Table 7 shows 56.1% of the C/C, 64% of the C/T, and 60% of the T/T genotype to have Gleason score values greater than 7. However, this was not statistically significant.

4. Discussion

PCa is a heterogenous disease which requires proper stratification of the patients according to the risk. TMPRSS2−ERG gene fusion is one such marker that has emerged in recent times with potential diagnostic and prognostic implications in PCa because of its prevalence. The TMPRSS2−ERG fusion has also been studied with response to therapy and outcomes. Evidence has suggested that TMPRSS2−ERG fusion−positive tumors may have longer PCa survival after androgen deprivation therapy. Reig et al[21] have shown that detection of TMPRSS2−ERG in metastatic-resistant PCa predicts resistance to docetaxel.

In the present study on Indian patients with PCa, of the 102 samples that could be scored for TMPRSS2−ERG fusion status, 26% of the samples were found to be positive for TMPRSS2−ERG fusion. Among the fusion-positive cases along with the deletion and translocation patterns, a new pattern of translocation followed by insertion was also found. This finding was in accordance with that of Ribeiro et al[20] who found similar rearrangement patterns of TMPRSS2−ERG genes.

The frequency of ERG-positive tumors in our study (26%) is similar to that in the study by Rawal et al[22] who have reported a low frequency; eight cases were ERG positive (27%) by FISH in a total cohort of 30 samples from a single medical institution in North India. However, Ateeq et al[23] detected ERG overexpression by IHC in 46 of 94 (48.9%) PCa cases from northern India; this was subsequently confirmed in a much smaller subset (17 samples) by FISH.

The frequency of TMPRSS2−ERG fusion is lower in the Indian population as indicated by our study compared with the Caucasian population[8] where 35.5% of PCa patients were positive for TMPRSS2−ERG but higher than that seen in a Korean cohort[24] where TMPRSS2−ERG positivity was only 20.9%. Interestingly, Peterson et al[5] in their meta-analysis found TMPRSS2−ERG fusion status to be 23% in Asian cohorts and roughly 50% in European and North American cohorts.

The differences in the frequency have been attributed to several factors, such as varying distributions of genetic or lifestyle factors associated with the risk of developing fusion-negative versus fusion-positive PCa. Moreover, the methodology for detecting TMPRSS2−ERG fusion has also been implicated with higher prevalence of fusion, with the highest in studies using RT-PCR (52%) or IHC (52%) to assess TMPRSS2−ERG fusion status, relative to studies using FISH (42%).[5] The advantages of using FISH as the technology is that it allows categorizing the type of fusion and enabling further delineation of PCa into subtypes which may have prognostic implications.

In the present study of the 27 samples positive for TMPRSS2−ERG, nine (33%) evidenced fusion by translocation which is lower than that found in the cohort [38/74 (50%)] evaluated by FitzGerald[8] but much higher than that (6.25%) seen in the study by Ribeiro et al.[20]

It may be again mentioned that Ribeiro et al[20] found fusion by insertion in 62.5% (10/16) of the cases; fusion by insertion has also

Table 1
General characteristics of PCa patients.

Characteristics	Total (n = 150)	Fusion positive (n = 27)	Fusion negative (n = 75)	P
Age of diagnosis (mean)	70.73	71.5	70.5	
Gleason score[a]				
2−7 (3 + 4)	59 (40.7%)	9 (36%)	32 (43.24%)	
7 (4 + 3)−10	86 (59.3%)	16 (64%)	42 (56.75%)	0.525
Perineural invasion[b]				
Not seen	52 (42.6%)	8 (38%)	25 (40.32%)	
Seen	70 (57.4%)	13 (62%)	37 (59.67%)	0.875
% tumor occupied[c]				
0−20	12 (8.9%)	—	6 (8.8%)	
20−40	14 (10.44%)	4 (16%)	8 (11.8%)	
40−60	21 (15.7%)	2 (8%)	15 (22%)	
60−70	21 (15.7%)	5 (20%)	12 (17.64%)	
70−80	37 (27.6%)	5 (20%)	17 (25%)	
80−90	22 (16.4%)	6 (24%)	9 (13.2%)	
>90	6 (4.5%)	3 (12%)	1 (1.47%)	

PCa, prostate cancer.
[a] Gleason score values were available for 145 of the 150 samples.
[b] Perineural invasion data were available for 122 of the 150 samples.
[c] Tumor % values were available for 134 of the 150 samples.

Table 2
Frequency of TMPRSS2–ERG fusion status and its clinical data.

Clinicopathological details	Fusion negative (n = 75)	Fusion positive (n = 27)			P (Chi-square test)
		Deletion (n = 6)	Translocation (n = 9)	Insertion (n = 12)	
Gleason score[a]					
2–7 (3 + 4)	32 (43.24%)	2 (40%)	3 (33.33%)	4 (36.36%)	0.96
7 (4 + 3)–10	42 (56.75%)	3 (60%)	6 (66.66%)	7 (63.63%)	
Perineural invasion[b]					
Not seen	25 (40.32%)	2 (50%)	3 (37.5%)	3 (33.33%)	0.849
Seen	37 (59.67%)	2 (50%)	5 (62.5%)	6 (66.66%)	
% tumor occupied[c]					
0–20	6 (8.8%)	—	—	—	
20–40	8 (11.8%)	1 (20%)	3 (33.33%)	—	
40–60	15 (22%)	1 (20%)	—	1 (9.1%)	
60–70	12 (17.64%)	1 (20%)	1 (11.11%)	3 (27.3%)	
70–80	17 (25%)	1 (20%)	1 (11.11%)	3 (27.3%)	
80–90	9 (13.2%)	—	4 (44.44%)	2 (18.2%)	
>90	1 (1.47%)	1 (20%)	—	2 (18.2%)	

[a] Gleason score values were available for 145 of the 150 samples.
[b] Perineural invasion data were available for 122 of the 150 samples.
[c] Tumor % values were available for 134 of the 150 samples.

Table 3
Association of TMPRSS2–ERG fusion status with Gleason pattern.

No	Gleason score	TMPRSS2 positive pts[a]	TMPRSS2 negative pts
1	3 + 3	3 (11.5%)	13 (17%)
2	3 + 4	5 (19%)	21 (28%)
3	3 + 5	2 (8%)	4 (5%)
4	4 + 3	5 (19%)	11 (15%)
5	4 + 4	5 (19%)	8 (11%)
6	4 + 5	1 (4%)	5 (7%)
7	5 + 3	3 (11.5%)	2 (3%)
8	5 + 4	0	6 (8%)
9	5 + 5	2 (%)	5 (6%)
	Total	26	75

FISH, fluorescence in situ hybridization.
[a] 27 FISH positive, Gleason score not available for 1.

Table 4
Genotypic and allelic frequency of rs12329760.

rs12329760	C/C	C/T	T/T	C	T
Total (n = 150)	84 (56%)	50 (33.3%)	16 (10.66%)	0.73	0.27
Fusion positive (n = 27)	12 (44.44%)	10 (37%)	5 (18.5%)	0.63	0.37
Fusion negative (n = 75)	47 (62.66%)	20 (26.66%)	8 (10.66%)	0.76	0.24

Table 5
Association of rs12329760 with fusion status.

rs12329760	OR	CI (95%)	P
C/C vs. T/T	2.448	0.847–6.643	0.072
C/C vs. C/T	1.750	0.364–4.310	0.783
C/C vs. C/T + T/T	2.176	1.012–4.684	0.040

Calculation was done using Chi-square test.
CI, confidence interval; OR, odds ratio.

been documented in the present study albeit with a lower frequency 44% (12/27). Fusion by deletion was lower in our cohort 22% (6/27) than the 50% seen in the study by FitzGerald[8] and by Fernandez et al[2] wherein 55% of PCa carried the TMPRSS2–ERG fusion gene of which 40% showed a split signal pattern and 12% showed an interstitial deletion. Notably, it has been observed that fusion formed via deletion, rather than translocation, to be associated with aggressive disease in an autopsy cohort of androgen-independent PCa.[14] Another study has reported that the presence

of TMPRSS2–ERG, resulting from an interstitial deletion accompanied by a high copy number of this gene (the so called class 2 + Edel), is associated with a poor prognosis,[25] but this has not been addressed by other studies. The probable reason attributed to gene fusions occurring by deletions having a worse prognosis than translocation is the 3-Mb region getting deleted which houses influential tumor suppressor genes.[13]

In the present study, no statistically significant difference was seen between the TMPRSS2–ERG rearrangement types and their clinical characteristics (P > 0.05). The TMPRSS2–ERG fusion was detected marginally more in patients with Gleason score in the range of ≥7 (4 + 3) with frequency of 62%. This is higher than that seen by Rajput et al (40.7%) in their study from 196 PCa cases using FISH;[26] their study also specified fusion status to be significantly higher in moderate to poorly differentiated tumor cells. Our study is in concordance with the findings of Perner et al[13] who did not find any association with Gleason score and those of Tavecku et al[27] who in their study on 40 patients undergoing radical prostatectomy and analyzed by RT-PCR did not find a significant correlation between TMPRSS2–ERG gene fusion status and tumor stage, Gleason grade, PSA level, and surgical margin status. The current findings are also similar to those observed in 63 prostate tumor specimens where the presence of a TMPRSS2–ERG fusion showed no statistical association with Gleason grade, in addition to survival or recurrence-free tumor stage.[28]

In contrast to our findings, several other studies and a meta-analysis,[6] positive statistical correlations were identified between TMPRSS2–ERG fusion, high s-PSA, pathological stage, and Gleason score by Rostad et al from a study on 55 patients analyzed for the presence of TMPRSS2:ERG isoforms using real-time qPCR.[16] Significant association between TMPRSS2–ERG fusion-positive PCas and higher Gleason scores (7 and 8–10 vs. 4–6, P = 0.027) was also observed in a German cohort of 86 biopsy-proven PCa patients which could be scored by FISH.[17] Interestingly, Darnel et al[29] in their Canadian cohort of 163 patients found significantly higher occurrence of TMPRSS2–ERG fusion (P = 0.014) in Gleason pattern 3 versus Gleason pattern 4 (42% vs. 27%).

In the present study, we have also determined the frequency of TMPRSS2 SNP rs12329760 Met160Val and its association with the fusion status in PCa patients. The frequency of the variant allele (T) in our cohort is 0.27. This frequency is almost similar to that of the CEU-Utah residents with Northern and Western European ancestry population (0.23); Yoruba in Ibdan, Nigeria (0.25); and African

Table 6
Univariate logistic regression of rs12329760 with *TMPRSS2−ERG* rearrangement.

Model	Genotype	FISH = 0	FISH = 1	OR (95% CI)	P	AIC	BIC
a) Translocation							
Codominant	C/C	41 (59.4%)	5 (62.5%)	1	0.96	59.3	68.6
	C/T	20 (29%)	2 (25%)	0.82 (0.15−4.58)			
	T/T	8 (11.6%)	1 (12.5%)	1.10 (0.10−11.91)			
Dominant	C/C	41 (59.4%)	5 (62.5%)	1	0.88	57.3	64.4
	C/T-T/T	28 (40.6%)	3 (37.5%)	0.89 (0.19−4.05)			
Recessive	C/C-C/T	61 (88.4%)	7 (87.5%)	1	0.9	57.3	64.4
	T/T	8 (11.6%)	1 (12.5%)	1.17 (0.11−12.06)			
Overdominant	C/C-T/T	49 (71%)	6 (75%)	1	0.8	57.3	64.3
	C/T	20 (29%)	2 (25%)	0.80 (0.15−4.36)			
Log additive	—	—	—	0.97 (0.33−2.90)	0.96	57.3	64.4
b) Insertion							
Codominant	C/C	41 (59.4%)	5 (41.7%)	1	0.54	74.1	83.7
	C/T	20 (29%)	5 (41.7%)	2.13 (0.55−8.28)			
	T/T	8 (11.6%)	2 (16.7%)	1.73 (0.27−11.19)			
Dominant	C/C	41 (59.4%)	5 (41.7%)	1	0.27	72.1	79.3
	C/T-T/T	28 (40.6%)	7 (58.3%)	2.00 (0.57−6.98)			
Recessive	C/C-C/T	61 (88.4%)	10 (83.3%)	1	0.78	73.3	80.5
	T/T	8 (11.6%)	2 (16.7%)	1.29 (0.22−7.52)			
Overdominant	C/C-T/T	49 (71%)	7 (58.3%)	1	0.33	72.4	79.6
	C/T	20 (29%)	5 (41.7%)	1.90 (0.53−6.86)			
Log additive	—	—	—	1.46 (0.64−3.35)	0.38	72.6	79.8
c) Interstitial deletion							
Codominant	C/C	41 (59.4%)	1 (16.7%)	1	0.11	45.3	54.5
	C/T	20 (29%)	3 (50%)	6.14 (0.60−62.82)			
	T/T	8 (11.6%)	2 (33.3%)	10.53 (0.78−142.05)			
Dominant	C/C	41 (59.4%)	1 (16.7%)	1	0.04	43.5	50.5
	C/T-T/T	28 (40.6%)	5 (83.3%)	7.27 (0.80−65.95)			
Recessive	C/C-C/T	61 (88.4%)	4 (66.7%)	1	0.19	46	53
	T/T	8 (11.6%)	2 (33.3%)	3.97 (0.56−28.28)			
Overdominant	C/C-T/T	49 (71%)	3 (50%)	1	0.28	46.6	53.5
	C/T	20 (29%)	3 (50%)	2.57 (0.47−14.10)			
Log additive	—	—	—	3.15 (0.98−10.07)	0.046	43.8	50.7

AIC, Akaike information criterion; BIC, Bayesian information criterion; CI, confidence interval; FISH, flourescence *in situ* hybridization; OR, odds ratio.

ancestry in Southwest USA (0.27) but lower than that found in the Japanese in Tokyo, Japan (0.40) (www.hapmap). More importantly, the frequency in the PCa cohort in the present study is higher than that seen in the normal GIH- Gujarati Indians from Houston, Texas (0.14). The comparison of normal Indians from the HapMap database with the Indian PCa cohort from the present study indicates that this difference is statistically significant ($\chi^2 = 7.88$, $P = 0.005$). Recently, the Met160Val has been also identified as a genetic risk factor for sporadic PCa in a Japanese population,[18] with T allele frequency in the control group being 0.372 and in the sporadic PCa cases being 0.435. It would be interesting to determine if the same holds true even for the Indian population with a case−control association study. In another case−control study,[30] however, it is seen that men with the GG genotype (reverse strand) and a first-degree family history of PCa had a significantly higher risk for PCa relative to men without a family history [odds ratio (OR) = 2.05; 95% confidence interval (CI) = 1.3−3.2]. However, the interaction between genotype and family history of PCa was not significant ($P = 0.52$).

Regarding the association of Met160Val with *TMPRSS2−ERG* fusion, our findings indicate an association between the 'T' allele

and increased risk of *TMPRSS2−ERG* fusion (OR = 2.176, 95% CI = 1.012−4.684, $P = 0.04$). In the study by Fitzgerald et al,[8] wherein four SNPs in the ERG gene (rs1571704, rs1892570, rs2068967, and rs2836370) and one SNP in the *TMPRSS2* gene (rs12329760) were evaluated with fusion status, none were associated with fusion status, but men with a variant T allele were more likely to have fusion by translocation (χ^2 test: $P = 0.05$) and to have multiple copies of the gene fusion (χ^2 test: $P = 0.03$). In our study, a positive correlation between *TMPRSS2−ERG* rearrangement by deletion and the polymorphism in the dominant model was seen (OR = 7.27, 95% CI = 0.80−65.95, $P = 0.04$), whereas Fitzgerald et al[8] have documented association with translocation.

In conclusion, this study on a fairly large cohort of Indian PCa patients revealed *TMPRSS2−ERG* fusion in 26% (27/102) of the samples; no association between *TMPRSS2−ERG* fusion and its clinical characteristics was seen, and an association of the rs12329760 SNP with *TMPRSS2−ERG* fusion, more specifically with interstitial deletion, was seen.

The limitation of the present study is the lack of follow-up data for these patients. Future studies are warranted in this population with follow-up data that would help correlate fusion status with clinical outcomes. Nevertheless, the present study yields valuable information on the frequency of *TMPRSS2−ERG* fusion and the *TMPRSS2* SNP Met160Val and determines for the first time the correlation between them, thus providing novel information in the Indian cohort of PCa patients.

Table 7
Distribution and association of genotype based on Gleason score values.

Genotype	Gleason score		OR (95% CI)	P
	2−7 (3 + 4)	7 (4 + 3)−10		
C/C (n = 84)	36 (43.9%)	46 (56.1%)	[a] 1.174 (0.498−2.792)	0.842
C/T (n = 50)	17 (35.4%)	31 (64.6%)	[b] 1.472 (0.698−2.937)	0.322
T/T (n = 16)	6 (40%)	9 (60%)	[c] 1.322 (0.734−2.389)	0.333

CI, confidence interval; OR, odds ratio.
[a] OR, 95% CI, C/C vs. T/T.
[b] OR, 95% CI, C/C vs. C/T.
[c] OR, 95% CI, C/C vs. C/T + T/T.

Conflicts of interest

All authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The SRL Ethics Committee reviewed, discussed, and approved the study.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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