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ERG oncoprotein expression in prostate carcinoma patients of different ethnicities

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Abstract. Overexpression of the erythroblast transformation-specific-related gene (ERG) oncoprotein due to transmembrane protease, serine 2 (*TMPRSS2*)-*ERG* fusion, the most prevalent genomic alteration in prostate cancer (CaP), is more frequently observed among Caucasian patients compared to patients of African or Asian descent. To the best of our knowledge, this is the first study to investigate the prevalence of *ERG* alterations in a multiethnic cohort of CaP patients. A total of 191 formalin-fixed paraffin-embedded sections of transrectal ultrasound-guided prostate biopsy specimens, collected from 120 patients treated at the Sime Darby Medical Centre, Subang Jaya, Malaysia, were analyzed for ERG protein expression by immunohistochemistry using the anti-ERG monoclonal antibody 9FY as a surrogate for the detection of *ERG* fusion events. The overall frequency of ERG protein expression in the population evaluated in this study was 39.2%. Although seemingly similar to rates reported in other Asian communities, the expression of ERG was distinct amongst different ethnic groups (P=0.004). Malaysian Indian (MI) patients exhibited exceedingly high expression of ERG in their tumors, almost doubling that of Malaysian Chinese (MC) patients, whereas ERG expression was very low amongst Malay patients (12.5%). When collectively analyzing data, we observed a significant correlation between younger patients and higher ERG expression (P=0.04). The prevalence of ERG expression was significantly different amongst CaP patients of different ethnicities. The higher number of ERG-expressing tumors among MI patients suggested that the *TMPRSS2-ERG* fusion may be particularly important in the pathogenesis of CaP amongst this group of patients. Furthermore, the more frequent expression of ERG among the younger patients analyzed suggested an involvement of ERG in the early onset of CaP. The results of this study underline the value of using ERG status to better understand the differences in the etiology of CaP initiation and progression between ethnic groups.

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Introduction

Prostate carcinoma (CaP) is the fourth most frequently diagnosed cancer among Malaysian men, preceded by lung, colorectal and nasopharyngeal cancers (1). However, consistent with the global tendency of an increasing median age of patient populations due to increased overall longevity, the incidence of CaP in Malaysia is also on the increase (2). Among the major ethnic groups in Malaysia, the incidence of CaP was found to increase after the age of 45 years and is highest among Malaysian Chinese (MC) compared to Malaysian Indian (MI) and Malay men (1).

There is a clear disparity in CaP incidence and mortality worldwide and the major determining factors are being actively investigated. Although socioeconomic status and access to healthcare are often associated with disparities in the diagnosis, treatment and survival of CaP patients of different ethnic backgrounds, contributing genetic differences have also been identified (3,4). Some of the tools used to characterize gene alterations and identify potential driver genes include genome-wide association studies, karyotyping of chromosomal copy number, as well as exome and whole-genome sequencing. In addition to the search for underlying genetic events that initiate cancer or distinguish aggressive from indolent tumors, the search for genetic alterations that may explain the ethnic disparities in CaP is currently actively pursued (3,5,6).

The variant allele on 8q24, which increases the risk for CaP, particularly in men of African ancestry, is one of the most convincing risk alleles for CaP (7,8). Another allele associated with an increased risk of CaP men of African ancestry is the rs743572 single-nucleotide polymorphism of *CYP17* (9).

More recently, evaluations of the transmembrane protease, serine 2 (*TMPRSS2*)-erythroblast transformation-specific (ETS)-related gene (*ERG*) fusion in different populations have highlighted the differences in frequency between ethnic groups. Recurrent gene fusions between regulatory sequences of an androgen receptor (AR)-regulated gene, such as *TMPRSS2*, solute carrier family 45 member 3 or N-myc downstream-regulated gene 1, and an *ETS* gene family member, such as *ERG*, *ETS* translocation variant (*ETV*)*1*, *4* and *5* as the 3' fusion partner, result in androgen-dependent expression of ETS transcription factors. Among these genetic alterations, the *TMPRSS2-ERG* fusion, detected in 50-70% of CaP patients from Western countries, is the most prevalent (10,11). The frequency of *TMPRSS2-ERG* gene fusions detected in CaPs of African Americans (AA) (31-43%) is often lower compared to that of Caucasian Americans (CA) (50-66%) (5,12). Interestingly, ERG overexpression is more frequently detected in the index tumors of CA (63.3%), compared to those of AA patients (28.6%) (5). However, evaluations of *TMPRSS2-ERG* gene fusions, either by immunohistochemistry (IHC) detection of ERG expression alone or in combination with fluorescence *in situ* hybridization (FISH), in different populations worldwide demonstrated lower frequencies compared to that detected CA and Europeans (12-20).

The aberrant overexpression of an ERG oncoprotein as a result of *TMPRSS2-ERG* fusion exerts a profound effect on cellular pathways associated with cancer initiation and progression (10,21-24). Evidence of the association between ERG-positive prostatic intraepithelial neoplasia lesions and ERG-positive prostate tumors highlights the significance of ERG activation in the early stages of tumor development (25). ERG overexpression inhibits prostate epithelial differentiation, while promoting epithelial-to-mesenchymal transition (26,27). In addition, ERG regulates target genes with functions in DNA damage repair, epigenetic silencing and inflammation, which affect pathways associated with tumor cell growth, proliferation and invasion (24). For example, the cooperation of ERG with phosphatase and tensin homolog (PTEN) deletion and activation of AKT has been shown to promote neoplastic transformation (28,29). A better understanding of how ERG interacts with cancer genes that contribute to cancer progression has led to the development of various treatment strategies that target ERG and its downstream effectors (30). The ability to clearly detect ERG expression in prostate tumors in contrast to normal glands by IHC using specific monoclonal antibodies (MAbs) has improved the diagnosis of the majority of CaPs (25,31). The high concordance between the evaluations of *TMPRSS2-ERG* fusion by FISH and ERG protein expression by IHC supports the reliability and accuracy of ERG IHC as a surrogate for FISH detection (25,31-33). Furthermore, the evaluation of prostate tumors for ERG expression, together with PTEN deletion and integrity of AR signaling pathways, may help the prognostic stratification of patients and the selection of treatment options (34,35).

To date, no study has evaluated the frequency of ERG alterations in CaP patients in Malaysia, which has a population comprising diverse ethnic groups. The major ethnic groups in Malaysia are Malays (55%), Chinese (24%) and Indians (7.2%) (36). In order to better understand the role of ERG in the etiology of CaP initiation and progression, we used the detection of ERG by IHC as a surrogate for *ERG* fusion events to evaluate the prevalence of ERG expression in a multiethnic cohort of Malaysian CaP patients.

Table I. Demographics of prostate cancer patients (n=120) and Gleason scores of tumor specimens (n=191).

Variables	No.	%
Age (years)		
<69	60	50.0
≥69	60	50.0
Ethnicity		
Chinese	82	68.3
Malay	8	6.7
Indian	30	25.0
Sections from right lobe (n=100)		
Gleason scores		
≤6	32	32.0
7 (3+4)	18	18.0
7 (4+3), 8-10	50	50.0
Sections from left lobe (n=91)		
Gleason scores		
≤6	27	29.7
7 (3+4)	20	22.0
7 (4+3), 8-10	44	48.3
Total prostate sections (n=191)		
Total Gleason scores		
≤6	59	30.9
7 (3+4)	38	19.9
7 (4+3), 8-10	94	49.2

Materials and methods

Specimens. Transrectal ultrasound (TRUS)-guided biopsies were performed on 120 patients who were diagnosed with CaP based on clinical findings at the Sime Darby Medical Centre, Subang Jaya, Malaysia. The specimens were collected between 2011 and 2013, following approval by an independent Ethics Committee of Sime Darby Healthcare (ethics reference no. 201309.5). The TRUS-guided biopsy entails targeting the suspected prostatic lesion, as well as random sampling of the prostatic gland. Typically, 12-18 biopsy cores were collected from each prostate. Occasionally, 24-36 biopsy cores were collected from patients with significantly larger prostates, or from whom a second biopsy was required. The biopsy specimens were fixed with formalin and embedded in paraffin blocks.

IHC detection for ERG expression. Anti-ERG-MAb 9FY was obtained (cat. no. CM421C; Biocare Medical, Concord, CA, USA). Sections (4-μm) were cut from formalin-fixed paraffin-embedded (FFPE) blocks, mounted on slides and deparaffinized. IHC was performed using a Ventana Benchmark Ultra autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) using Ventana reagents. Briefly, the

No	Patient	Left Lobe	Gleason Sum (Left)	Right Lobe	Gleason Sum (Right)	Overall ERG Status	Highest Gleason	No	Patient	Left Lobe	Gleason Sum (Left)	Right Lobe	Gleason Sum (Right)	Overall ERG Status	Highest Gleason
1	MC1		NS		3+3=6	-	6	61	MC61		4+5=9		4+5=9	-	9
2	MC2		NS		3+3=6	-	6	62	MC62		5+4=9		5+4=9	-	9
3	MC3		NS		3+3=6	-	6	63	MC63		5+3=8		5+5=10	-	10
4	MC4		NS		3+3=6	-	6	64	MC64		3+3=6		3+3=6	+	6
5	MC5		NS		3+4=7	-	7	65	MC65		3+3=6		4+5=9	+	9
6	MC6		NS		3+4=7	-	7	66	MC66		4+5=9		4+5=9	+	9
7	MC7		NS		4+3=7	-	7	67	MC67		2+3=5		2+3=5	+	5
8	MC8		NS		4+4=8	-	8	68	MC68		4+3=7		4+3=7	+	7
9	MC9		NS		4+4=8	-	8	69	MC69		3+4=7		3+4=7	+	7
10	MC10		NS		4+5=9	-	9	70	MC70		3+3=6		3+3=6	+	6
11	MC11		NS		2+3=5	+	5	71	MC71		3+3=6		3+3=6	+	6
12	MC12		NS		3+3=6	+	6	72	MC72		3+4=7		3+4=7	+	7
13	MC13		NS		3+3=6	+	6	73	MC73		4+4=8		4+4=8	+	8
14	MC14		NS		4+3=7	+	7	74	MC74		4+5=9		3+5=8	+	9
15	MC15		NS		4+5=9	+	9	75	MC75		3+3=6		3+3=6	+	6
16	MC16		3+3=6		NS	-	6	76	MC76		4+4=8		3+4=7	+	8
17	MC17		3+3=6		NS	-	6	77	MC77		4+5=9		4+5=9	+	9
18	MC18		3+3=6		NS	-	6	78	MC78		3+3=6		3+3=6	+	6
19	MC19		3+3=6		NS	-	6	79	MC79		3+4=7		3+3=6	+	7
20	MC20		3+4=7		NS	-	7	80	MC80		3+4=7		3+4=7	+	7
21	MC21		3+4=7		NS	-	7	81	MC81		4+4=8		4+4=8	+	8
22	MC22		3+4=7		NS	-	7	82	MC82		4+5=9		4+5=9	+	9
23	MC23		3+4=7		NS	-	7	83	MI1		NS		3+3=6	-	6
24	MC24		4+3=7		NS	-	7	84	MI2		NS		3+3=6	-	6
25	MC25		4+4=8		NS	-	8	85	MI3		NS		3+3=6	-	6
26	MC26		4+4=8		NS	-	8	86	MI4		NS		3+4=7	-	7
27	MC27		3+3=6		NS	+	6	87	MI5		NS		5+4=9	-	9
28	MC28		3+3=6		NS	+	6	88	MI6		NS		4+5=9	-	9
29	MC29		3+4=7		NS	+	7	89	MI7		NS		3+3=6	+	6
30	MC30		2+3=5		2+3=5	-	5	90	MI8		NS		3+4=7	+	7
31	MC31		2+3=5		2+3=5	-	5	91	MI9		NS		3+4=7	+	7
32	MC32		3+2=5		3+2=5	-	5	92	MI10		NS		3+4=7	+	7
33	MC33		2+3=5		2+3=5	-	5	93	MI11		NS		3+5=8	+	8
34	MC34		3+4=7		2+3=5	-	7	94	MI12		NS		4+4=8	+	8
35	MC35		4+4=8		3+2=5	-	8	95	MI13		NS		4+5=9	+	9
36	MC36		3+3=6		3+3=6	-	6	96	MI14		3+4=7		NS	-	7
37	MC37		3+3=6		3+3=6	-	6	97	MI15		3+3=6		NS	+	6
38	MC38		3+3=6		3+3=6	-	6	98	MI16		3+4=7		NS	+	7
39	MC39		3+3=6		3+3=6	-	6	99	MI17		4+3=7		NS	+	7
40	MC40		3+3=6		3+3=6	-	6	100	MI18		3+4=7		NS	+	7
41	MC41		3+3=6		3+3=6	-	6	101	MI19		3+4=7		3+4=7	-	7
42	MC42		3+3=6		3+4=7	-	7	102	MI20		4+4=8		4+4=8	-	8
43	MC43		3+4=7		3+4=7	-	7	103	MI21		4+4=8		4+4=8	-	8
44	MC44		4+3=7		4+3=7	-	7	104	MI22		5+3=8		5+4=9	-	9
45	MC45		3+4=7		3+4=7	-	7	105	MI23		3+4=7		3+4=7	+	7
46	MC46		3+4=7		3+4=7	-	7	106	MI24		4+3=7		4+3=7	+	7
47	MC47		4+4=8		4+4=8	-	8	107	MI25		4+5=9		4+5=9	+	9
48	MC48		4+4=8		4+4=8	-	8	108	MI26		3+4=7		3+3=6	+	7
49	MC49		4+4=8		4+4=8	-	8	109	MI27		5+4=9		3+4=7	+	9
50	MC50		4+4=8		4+4=8	-	8	110	MI28		5+4=9		5+4=9	+	9
51	MC51		3+3=6		5+4=9	-	9	111	MI29		4+5=9		4+5=9	+	9
52	MC52		5+4=9		5+4=9	-	9	112	MI30		4+5=9		4+5=9	+	9
53	MC53		5+4=9		5+4=9	-	9	113	MAL1		NS		2+3=5	-	5
54	MC54		4+5=9		4+5=9	-	9	114	MAL2		5+4=9		NS	-	9
55	MC55		4+5=9		4+5=9	-	9	115	MAL3		2+2=4		4+3=7	-	7
56	MC56		4+5=9		4+5=9	-	9	116	MAL4		3+4=7		3+4=7	-	7
57	MC57		4+5=9		4+5=9	-	9	117	MAL5		4+4=8		4+3=7	-	8
58	MC58		4+5=9		4+5=9	-	9	118	MAL6		4+4=8		4+4=8	-	8
59	MC59		4+5=9		4+5=9	-	9	119	MAL7		4+5=9		4+5=9	-	9
60	MC60		4+5=9		4+5=9	-	9	120	MAL8		5+4=9		5+4=9	+	9

Figure 1. Erythroblast transformation-specific-related gene (ERG) oncoprotein expression of tumor sections from each patient examined. Heatmap representation of ERG expression in both patients and sections. Green, no expression; pink, low expression; peach, moderate expression; dark red, strong expression. Grey, no sections evaluated. MC, Malaysian Chinese; NS, not scored; MI, Malaysian Indian; MAL, Malay.

sectioned specimens were processed for antigen retrieval using CC1 antigen retrieval solution prediluted in Tris/borate/EDTA buffer (pH 8.0-8.5) and incubated at 95˚C for 48 min. The sections were then put through peroxidase inhibition prior to incubation with ERG-MAb at a dilution of 1:100 for 20 min at room temperature. ERG expression was detected by using OptiView HQ universal Linker and OptiView HRP Multimer (Ventana Medical Systems, Inc.), incubated consecutively at

Table II. Association of erythroblast transformation-specific-related gene (ERG) oncoprotein expression status with ethnicity, age and Gleason score, as evaluated by patient and by individual tumor sections.

	Evaluation by patient (n=120)			Evaluation by individual tumor sections (n=191)		
	ERG expression			ERG expression		
Variables	Negative (%)	Positive (%)	P-value	Negative (%)	Positive (%)	P-value
Ethnicity			0.004[a,b]			<0.001[a]
Chinese	55 (67.1)	27 (32.9)		100 (74.1)	35 (25.9)	
Malay	7 (87.5)	1 (12.5)		13 (92.9)	1 (7.1)	
Indian	11 (36.7)	19 (63.3)		18 (42.9)	24 (57.1)	
Total	73 (60.8)	47(39.2)		131 (68.6)	60 (31.4)	
Age (years)			0.040[a,c]			0.015[a,c]
<69	31 (51.7)	29 (48.3)		56 (60.2)	37 (39.8)	
≥69	42 (70.0)	18 (30.0)		75 (76.5)	23 (23.5)	
Gleason score			0.813[c]			0.476[c]
≤6	22 (62.9)	13 (37.1)		41 (69.5)	18 (30.5)	
7 (3+4)	15 (55.6)	12 (44.4)		23 (60.5)	15 (39.5)	
7 (4+3), 8-10	36 (62.1)	22 (37.9)		67 (71.3)	27 (28.7)	

[a]P<0.05 (statistically significant difference). Data were analyzed by [b]Kruskal-Wallis test or [c]Pearson's Chi-square test.

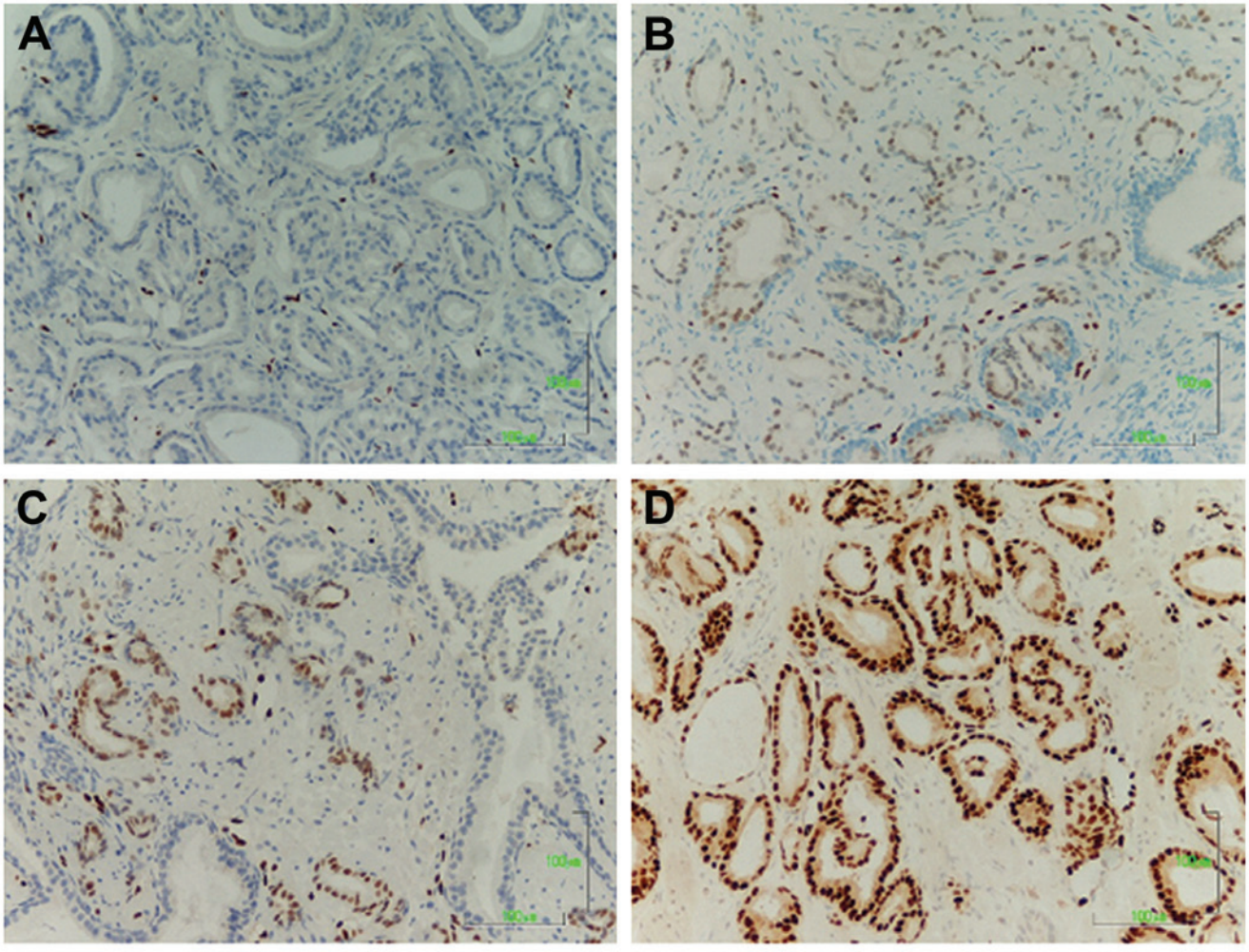

Figure 2. Immunohistochemical staining for the expression of erythroblast transformation-specific-related gene (ERG) oncoprotein using anti-ERG-MAb 9FY. Representative images showing (A) 0, negative; (B) 1+, mildly positive; (C) 2+, moderately positive; and (D) 3+, strongly positive staining for ERG expression.

Table III. Association of erythroblast transformation-specific-related gene (ERG) oncoprotein staining intensity in the examined sections with ethnicity, age and Gleason score.

	ERG staining intensity (%)			
Variables	Negative (0)	Weak (1+)	Strong (2+ and 3+)	P-value
Ethnicity				<0.001[a,b]
Chinese	100 (74.1)	7 (5.2)	28 (20.7)	
Malay	13 (92.9)	0 (0.0)	1 (7.1)	
Indian	18 (42.9)	7 (16.6)	17 (40.5)	
Age (years)				0.032[a,c]
<69	56 (60.2)	7 (7.5)	30 (32.3)	
≥69	75 (76.5)	7 (7.2)	16 (16.3)	
Gleason score				0.397[b]
≤6	41 (69.5)	7 (11.9)	11 (18.6)	
7 (3+4)	23 (60.5)	2 (5.3)	13 (34.2)	
7 (4+3), 8-10	67 (71.3)	5 (5.3)	22 (23.4)	

[a]P<0.05 (statistically significant difference). Data were analyzed by [b]Kruskal-Wallis test or [c]Pearson's Chi-square test.

room temperature for 8 min. The color was developed using Bluing reagent for 4 min and the sections were counterstained with hematoxylin. The ERG protein expression status and Gleason scores of the prostate sections were evaluated by a trained pathologist. Depending on the amount and intensity of the ERG IHC staining, the specimens were scored as follows: 0, negative; 1+, mild; 2+, moderate; and 3+, strong staining. Positive staining of endothelial cells in the specimens served as a built-in control for the staining.

Statistical analysis. Statistical analyses were performed using SPSS 16.0 software for Windows (IBM, Inc., New York, NY, USA). The Pearson's Chi-square and Kruskal-Wallis tests were used to determine the statistical associations of ERG expression with ethnicity, age and Gleason sum score. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient demographics and Gleason score of prostate specimens. The ERG oncoprotein expression status was evaluated in tumor specimens from 120 patients by demographic distribution and tumor Gleason scores (Table I). The mean age of the patients was 69 years (range, 52-91 years). MC patients represented the largest ethnic group in this study (68.3%), followed by MI (25%) and Malay patients (6.7%). Both the left and right lobes of the prostate were biopsied in all the patients. Among the 120 patients, 71 were found to have tumors in both lobes, whereas 20 patients had tumors in only the left lobe and 29 had tumors in only the right lobe of the prostate. Specifically, of the 191 tumor sections that were evaluated, 91 were from the left lobe and 100 were from the right lobe of the prostate (Table I and Fig. 1). The ERG expression status for each patient was scored as positive if ERG oncoprotein expression was detected in tumor sections from either lobe, taking into account inter-tumoral heterogeneity within the same prostate (25).

Prevalence of ERG expression in different ethnic groups. The evaluation of ERG oncoprotein expression by IHC in the multiethnic cohort of Malaysian CaP patients revealed an overall frequency of 39.2%, with positive ERG expression in 47 of the 120 patients. ERG-positive tumors were detected in 31.4% (60/191) of the individual tumor sections examined. The status and intensity of ERG staining are detailed in the heatmap in Fig. 1 and sections representative for each level of expression are shown in Fig. 2.

Among the MC patients, who formed the majority ethnic group of this study, 27 of 82 cases (32.9%) were ERG-positive (Table II). Prostate tumor sections were evaluated from either the right or left lobe of the prostate in 29 cases and from both lobes in 53 cases (Fig. 1). Positive ERG expression was detected in 35 of the 135 sections (25.9%) examined (Table II). Of the 27 cases positive for ERG expression, ERG was detected in either the right or the left lobe of the prostate in 19 and in both lobes in 8 cases.

Surprisingly, 19 of 30 (63.3%) MI patients were positive for ERG expression (Table II). Biopsy specimens were examined from either the right or left lobe of the prostate in 18 and from both lobes in 12 cases. Positive ERG expression was detected in 24 of 42 (57.1%) individual tumor sections (Table II). Of the 19 MI patients with a positive ERG expression status, ERG was detected in either the right or the left lobe of the prostate in 14 patients and in both lobes in 5 cases.

Among the 8 Malay patients evaluated, only 1 (12.5%) was positive for ERG (Table II). Prostate tumor sections from both lobes of the prostate were evaluated for 6 of the 8 Malay patients. Only 1 of the 14 (7.1%) sections examined was positive for ERG expression (Table II).

Analysis of the association of the ERG expression status of patients with age and Gleason score. The association of ERG expression status of patients with age and Gleason score of tumors was evaluated by statistical analysis. The

Table IV. Summary of the frequency of erythroblast transformation-specific-related gene (ERG) oncoprotein expression status in different populations worldwide.

Population	Sample	Assay method for ERG detection	Frequency, % (no./total)	(Refs.)
USA				
NSeg	RP	FISH	41.6 (217/521)	(45)
CA	Biopsy	FISH	46.0 (46/100)	(46)
NSeg	RP, WM	IHC, FISH	65.1 (86/132)	(25)
CA	RP	FISH	50.0 (21/42)	(12)
AA	RP	FISH	31.3 (20/64)	(12)
CA	RP, WM	IHC, FISH	65.9 (60/91)	(5)
AA	RP, WM	IHC, FISH	42.9 (39/91)	(5)
UK	TURP	FISH	30.1 (134/445)	(37)
Sweden	TURP	FISH, RT-PCR	17.5 (62/354)	(47)
	TURP	FISH, RT-PCR	16.9 (46/272)	(48)
Germany	PCa, LNMets, Mets	IHC, FISH	45.3 (120/265)	(32)
	RP	FISH	58.7 (44/75)	(49)
Japan	RP	FISH	15.9 (7/44)	(12)
	RP	IHC	16.3 (15/92)	(17)
	RP and biopsy	IHC	20.1 (42/209)	(16)
	RP	RT-PCR	27.8 (54/194)	(15)
Korea	RP	FISH	20.9 (53/254)	(13)
	RP	IHC	24.4 (73/303)	(18)
China	NS	FISH	7.5 (7/93)	(14)
		IHC	10.2 (9/88)	(50)
	TURP	FISH	23.2 (44/190)	(20)
India	RP	IHC, FISH	26.7 (8/30)	(19)
Malaysia				
NSeg	TRUS-biopsy	IHC	39.2 (47/120)	Present study
MC			32.9 (27/82)	
MI			63.3 (19/30)	
Malay			12.5 (1/8)	

NS, not specified. NSeg, not segregated; RP, radical prostatectomy; FISH, fluorescence *in situ* hybridization; CA, Caucasian American; WM, whole-mounted prostate sections; IHC, immunohistochemistry; AA, African American; TURP, transurethral resection of the prostate; RT-PCR, reverse transcription-polymerase chain reaction; PCa, localized prostate cancer; LNMets, lymph node metastasis; Mets, metastasis; TRUS, transrectal ultrasound; MC, Malaysian Chinese; MI, Malaysian Indian.

results revealed a positive correlation between positive ERG expression of tumors and younger patients, when evaluated either by patient (P=0.04) or by individual tumor sections (P=0.015; Table II). We also observed a correlation between higher intensity of ERG staining with younger patients as a whole (P=0.032; Table III). The evaluation of the association between ERG expression status and Gleason score, either by patient or by individual tumor sections, did not reveal a significant correlation (Table II).

Discussion

In this study, we evaluated the expression of ERG oncoprotein in a multiethnic cohort of patients as a surrogate for the detection of *TMPRSS2-ERG* fusion events. We examined 191 sections of FFPE prostate tumor specimens isolated by TRUS-guided biopsy from 120 patients. The ethnic distribution of this study cohort, which consisted of 82 MC (68.3%), 30 MI (25.0%) and 8 Malay men (6.7%), is representative of patient enrollment at the hospital where this study was conducted and does not mirror the ethnic distribution of the overall Malaysian population. However, it does represent the overall incidence of CaP diagnosed in the country, with the highest incidence among MC, followed by MI, and the lowest among Malays (1).

The overall frequency of ERG oncoprotein expression in the cohort of Malaysian CaP patients, as determined by IHC, was 39.2%, which was considerably lower compared to the frequency of 50-70% detected in Western countries. The prevalence of ERG among MC, the largest ethnic group analyzed, was 32.9%. Although this frequency of

ERG-positive expression in the MC population is marginally higher compared to the frequencies of 15.9-29.7% reported for populations from Korea, Japan and China, it remains within a similar range (12,14,15,18,20) (Table IV).

Interestingly, we detected a disproportionately higher frequency (63.3%) of ERG-positive tumors among MI patients in this study. This is in comparison to a previous study on Indian CaP patients without prior hormonal treatment from New Delhi, India, in which ERG-positive tumors were detected in 8 of the 30 cases (27%) examined (19). However, the higher prevalence of ERG-positive cases in this study may be attributed to the limitations inherent in a small sample size. Whether the higher prevalence of ERG-positive tumors among MI patients indicates a regional variation where *TMPRSS2-ERG* fusion contributes more significantly to the progression of the disease compared to other populations of the same ethnicity, requires confirmation by studies on larger populations. Among the three ethnic groups, Malay CaP patients exhibited the lowest frequency (12.5%) of ERG-positive tumors. However, the results obtained from the small sample of Malay patients analyzed in this study require further confirmation in studies involving larger cohorts.

Efforts to identify the correlation of *TMPRSS2-ERG* fusion or ERG overexpression with clinicopathological characteristics have yielded variable results, which is likely due to the heterogeneity of patient cohorts evaluated in different studies. In certain studies, a higher Gleason score and a lower tumor differentiation exhibited a significant correlation with *ERG* gene alterations or with ERG-positive immunostaining (25,37-39). Other studies have reported the association of a lower Gleason score with a higher number of *TMPRSS2-ERG* fusion events (13,40). However, other studies have reported a significant association of *TMPRSS2-ERG* fusion with tumors of higher stage and lymph node metastasis (41) or higher pathological stage (42), but no association between *TMPRSS2-ERG* fusion and Gleason score. In a comparison between patients of different ethnic backgrounds, Rosen *et al* (5) reported a correlation between ERG-negative status and high-grade CaP tumors among AA but not among CA patients. There was no significant correlation between Gleason score and ERG expression or intensity when evaluated against either tumor sections or patients in our study. However, a significant association between younger patients (aged <69 years) and a positive ERG expression status, as well as ERG intensity, was observed in our Malaysian cohort as a whole. This correlation was also observed in studies among Japanese and European CaP patients (17,43), which suggests that ERG rearrangement may be particularly important in patients with early-onset CaP.

The effect of multiple factors, including diet, genetics and environmental factors, may contribute to the significant disparity in the frequency of CaP globally. The *TMPRSS2-ERG* gene fusion alteration, which is frequent among Western Caucasian populations, has been found to be less frequent among South Asian and East Asian populations. Whether other genomic alteration events typified by the fusion of other *ETS* gene family members, such as *ETV1* and *ETV4*, to androgen-regulated promoters (10,23), amplification of the 8q24 loci (44), PTEN deletion (20), or yet to be identified genetic events, are more prevalent in Asian populations remains to be investigated. A more comprehensive study, including a larger number of Malay and MI patients should be undertaken, not only to confirm the frequency of *TMPRSS2-ERG* fusion events, but to gain better understanding of the underlying genetics of CaP in the Malaysian population.

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