

Molecular cytogenetic analysis for *TFE3* rearrangement in Xp11.2 renal cell carcinoma and alveolar soft part sarcoma: validation and clinical experience with 75 cases

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Renal cell carcinoma with *TFE3* rearrangement at Xp11.2 is a distinct subtype manifesting an indolent clinical course in children, with recent reports suggesting a more aggressive entity in adults. This subtype is morphologically heterogeneous and can be misclassified as clear cell or papillary renal cell carcinoma. *TFE3* is also rearranged in alveolar soft part sarcoma. To aid in diagnosis, a break-apart strategy fluorescence *in situ* hybridization (FISH) probe set specific for *TFE3* rearrangement and a reflex dual-color, single-fusion strategy probe set involving the most common *TFE3* partner gene, *ASPSR1*, were validated on formalin-fixed, paraffin-embedded tissues from nine alveolar soft part sarcoma, two suspected Xp11.2 renal cell carcinoma, and nine tumors in the differential diagnosis. The impact of tissue cut artifact was reduced through inclusion of a chromosome X centromere control probe. Analysis of the UOK-109 renal carcinoma cell line confirmed the break-apart *TFE3* probe set can distinguish the subtle *TFE3/NONO* fusion-associated inversion of chromosome X. Subsequent extensive clinical experience was gained through analysis of 75 cases with an indication of Xp11.2 renal cell carcinoma ($n=54$), alveolar soft part sarcoma ($n=13$), perivascular epithelioid cell neoplasms ($n=2$), chordoma ($n=1$), or unspecified ($n=5$). We observed balanced and unbalanced chromosome X;17 translocations in both Xp11.2 renal cell carcinoma and alveolar soft part sarcoma, supporting a preference but not a necessity for the translocation to be balanced in the carcinoma and unbalanced in the sarcoma. We further demonstrate the unbalanced separation is atypical, with *TFE3/ASPSR1* fusion and loss of the derivative X chromosome but also an unanticipated normal X chromosome gain in both males and females. Other diverse sex chromosome copy number combinations were observed. Our *TFE3* FISH assay is a useful adjunct to morphologic analysis of such challenging cases and will be applicable to assess the growing spectrum of *TFE3*-rearranged tumors.

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Renal cell carcinoma with translocations involving chromosome Xp11.2 resulting in *TFE3* gene rearrangement has recently been recognized as a distinct entity in the World Health Organization renal tumor classification scheme.¹ Tumors of this renal cell carcinoma subtype are generally characterized by a papillary architecture composed of cells with voluminous clear or eosinophilic cytoplasm and

psammoma bodies;^{2,3} however, the histologic appearance can be variable and may resemble clear cell or papillary renal cell carcinoma, which creates diagnostic difficulties.^{4–6} Initial cases of Xp11.2-rearranged renal cell carcinoma were confined to children and young adults who generally presented at advanced stages but with a relatively indolent short-term clinical outcome.^{3,7} In contrast, adults are now known to be affected, and evidence is accumulating that *TFE3* rearrangement in adult renal cell carcinoma may have a more aggressive course.^{8–14}

The *TFE3* gene (transcription factor for immunoglobulin heavy-chain enhancer 3) at Xp11.2 is a member of the microphthalmia-associated (MITF)

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family of basic helix–loop–helix leucine zipper transcription factors.¹⁵ *TFE3* can fuse to multiple partners in Xp11.2 renal cell carcinoma; most commonly *ASPSCR1* (also called *ASPL*) resulting in a t(X;17)(p11.2;q25.3).^{5,16} The *TFE3/ASPSCR1* involves fusion of either exon 3 or 4 of *TFE3* to *ASPSCR1*, which replaces the N-terminus of *TFE3* but retains the DNA-binding region, activation domain, and nuclear localization signal.¹⁷ Other known partners of *TFE3* include *PRCC*,^{18–20} *PSF*,²¹ *NONO*,²¹ or *CLTC*²² resulting from t(X;1)(p11.2;q21), t(X;1)(p11.2;p34), inv(X)(p11.2q12), or t(X;17)(p11.2;q23), respectively. Each of these rearrangements provides a more robust promoter for *TFE3* causing overexpression of the chimeric fusion product and a subset are known to have a stronger capacity for transactivation of other genes.^{23,24}

The same *TFE3/ASPSCR1* fusion in Xp11.2 renal cell carcinoma is also observed in alveolar soft part sarcoma,¹⁷ and both tumor types share a resistance to adjuvant chemotherapy.^{6,25,26} Alveolar soft part sarcoma are mesenchymal tumors with a wide variation in the onset age and location.²⁷ Although alveolar soft part sarcomas are rare and slow growing with prolonged survival, the long-term disease-specific mortality is high because of distant metastasis years after the initial diagnosis, highlighting the necessity of an accurate identification.²⁵ Typical alveolar soft part sarcomas are histologically characterized by large and round-to-polygonal tumor cells with intracytoplasmic periodic acid–Schiff-positive crystalloid inclusions and eosinophilic granular or vacuolated cytoplasm that are arranged in a pseudoalveolar pattern due to loss of central cohesion and surrounded by fibrous septae.²⁷ Alveolar soft part sarcomas are generally straightforward to diagnose, although questions arise when cases present as a metastasis without a known primary or as a primary in an uncommon site such as bone, and/or with unusual morphologic features.

To address diagnostically challenging cases of alveolar soft part sarcoma and Xp11.2 renal cell carcinoma, consideration of clinical presentation and histopathologic features has historically been supplemented by immunohistochemistry (IHC) for *TFE3* protein; however, the antibody is limited in sensitivity and specificity,^{28–32} which may be influenced by the method employed.³³ A genetic approach is therefore required to confirm *TFE3* rearrangement. Chromosome analysis necessitates a fresh tissue sample, and rarely is such available. Reverse transcription PCR (RT-PCR) is technically challenging because of RNA degradation in paraffin-embedded tissues and also requires knowledge of all partner genes, some of which are currently not established for Xp11.2 renal cell carcinoma.^{9,34} Fluorescence *in situ* hybridization (FISH) does not necessitate knowledge of the fusion partners and is a highly sensitive method for analysis of paraffin-embedded tissues. In response, we demonstrate

through both validation and extensive clinical experience that a break-apart strategy probe set for *TFE3*, including a chromosome X centromere probe as a control and a *TFE3/ASPSCR1* dual-color, single-fusion reflex probe set, is an excellent test to aid in the identification of tumors with Xp11.2 rearrangement. We also describe the clinical experience with these probe sets in a large series of diagnostic cases, which is an important resource to understand the complications associated with evaluating *TFE3*.

Materials and methods

Clinical Materials

Following Institutional Review Board approval, formalin-fixed, paraffin-embedded tissues from nine alveolar soft part sarcoma cases and two suspected Xp11.2 rearranged renal cell carcinoma cases were obtained from Mayo Clinic tissue registry and consultation files. Tumors typically considered in the differential diagnosis were included to assess diagnostic specificity: three cases each of pheochromocytoma, typical renal cell carcinoma (ie, with pathology not suggestive of Xp11.2 rearrangement), and metastatic melanoma. A hematoxylin and eosin (H&E) stained slide from each tumor was assessed by a pathologist to delineate the region of interest. Non-neoplastic tissue from 25 females and 25 males was also analyzed as normal controls. Following test validation and implementation in the clinical laboratory, an additional 75 cases were studied including 54 renal cell carcinoma, 13 alveolar soft part sarcoma, two perivascular epithelioid cell neoplasms (PEComas), one chordoma, and five unspecified tumors.

Cell Line

The UOK-109 cell line, derived from a 39-year-old male's primary papillary renal cell carcinoma with inv(X)(p11.2q12), was provided by WM Linehan at the National Cancer Institute (Bethesda, MD, USA).³⁵ Cells were cultured in DMEM with 10% fetal bovine serum at 5% CO₂. Chromosome analysis was performed using standard procedures.

Probe Design

Bacterial artificial chromosomes were selected using the University of California Santa Cruz Biotechnology Genome Browser and Database (<http://genome.ucsc.edu>, March 2006, genome build hg18) and obtained from Invitrogen (Carlsbad, CA, USA). DNA was isolated from bacterial cultures following a standard protocol using a Qiagen Plasmid Maxi Kit (Qiagen, Valencia, CA, USA). The extracted DNA was fluorescently labeled via nick translation (Abbott Molecular, Des Plaines, IL, USA) and the specificity of each clone was confirmed by

hybridization to normal blood metaphases and PCR. The clones were then combined to create the two probe sets detailed below.

A probe set using a break-apart strategy was developed to identify rearrangements of *TFE3* at Xp11.2. This probe set includes a 3' *TFE3* telomeric probe, labeled in SpectrumGreen (Abbott Molecular), that consists of RP11-1137J13 and RP11-416B14 (273 kb total size). The 5' *TFE3* centromeric probe, labeled in Spectrum Orange (Abbott Molecular), which gives a red-appearing signal, consists of RP11-315L18 and RP11-1037C20 (416 kb total size). The proximity of the red and green signals in non-rearranged *TFE3* results in a yellow fusion signal. A commercially available chromosome X centromere probe (DXZ1), labeled in SpectrumAqua (Abbott Molecular), was also included as a control. This probe set was designed to detect both type 1 and type 2 *TFE3/ASPSCR1* transcripts, which based on current nomenclature involve fusion of exons 1–7 of *ASPSCR1* to exon 6 and exon 5 of *TFE3*, respectively.³⁶ Distinction of these two transcript types may be unnecessary as no differences in clinical setting, morphology, or behavior were observed,³⁷ although further studies are needed to confirm these findings.

A dual-color, single-fusion probe set, designated *ASPSCR1* reflex, was also developed to further assess cases showing an atypical rearrangement using break-apart *TFE3*. The purpose was to confirm the presence of the oncogenic fusion product of *TFE3* with *ASPSCR1* at 17q25.3. This probe set would also be critical to confirm cases of a suspected unbalanced *TFE3* rearrangement in males. The *ASPSCR1* reflex probe set consists of RP11-634L10 targeting *ASPSCR1* labeled in SpectrumOrange (172 kb total size), the 3' *TFE3* portion of the break-apart *TFE3* probe set (SpectrumGreen), and the chromosome X centromere (SpectrumAqua).

FISH

For each sample, 5- μ m thick formalin-fixed, paraffin-embedded tissue sections were placed on positively charged slides. The tumor region to be probed was then marked based on prior pathologist review of the matched H&E-stained section. The tissues were deparafinized at 90 °C, immersed twice in xylene and twice in 100% ethanol, air dried, and microwaved in 10 mM citric acid for 10 min. The slides were subsequently pretreated with 2xSSC for 5 min, digested with DigestALL (Invitrogen) at 37 °C for 40 min, and washed for 3 min in PBS. Serial dehydration then occurred by immersion in 70, 85 and then 100% ethanol for 3 min each at room temperature. Following drying, probe was applied to the marked area and co-denatured with the target DNA at 80 °C for 5 min and then hybridized at 37 °C overnight in a humidified incubator. After washing in NP40/2xSSC at 76 °C for 2 min, 10% DAPI counterstain (Abbott Molecular) was applied.

All validation samples were randomly sorted and analyzed in a blinded manner (without knowledge of the diagnosis) by two technologists who each independently scanned the entire tissue section to assess tumor heterogeneity and then scored 100 representative nuclei. In order to minimize scoring signal splitting due to artifact (ie, false positive separation), the criterion used for calling two signals as 'separate' was the presence of at least one signal width of space or the X centromere aqua signal in between the red and green signals. When the data were decoded, the non-neoplastic tissues were used to establish normal cutoff values for males and females for each signal pattern; specifically, the maximum number of false positive nuclei per signal pattern were identified and used in a binomial distribution to obtain the upper bound of the 95th percentile. For the break-apart *TFE3* probe set, the normal cutoff for *TFE3* rearrangement in males is <20% and in females is <11%, which reflects cut artifact inherent to paraffin tissue section analysis.

Each case demonstrating the atypical separation pattern with the break-apart *TFE3* probe set was also interrogated using the *ASPSCR1* reflex probe set to confirm *TFE3/ASPSCR1* fusion.

RT-PCR

RNA was extracted from formalin-fixed, paraffin-embedded blocks using the High Pure FFPE RNA Micro Kit (Roche Applied Science, Indianapolis, IN, USA) and converted into cDNA with random hexamers using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science). PCR reactions were carried out with Platinum Taq DNA Polymerase (Invitrogen) under the following conditions: 94 °C for 2 min; for 40 cycles, 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s; 72 °C for 7 min. The PCR primer sets employed were previously reported³⁷ and detect the following: *ASPSCR1/TFE3* type 1 and 2 fusions, the individual *ASPSCR1/TFE3* type 2 fusion plus the reciprocal products of *TFE3/ASPSCR1* type 1 and type 2 fusions, and the individual *TFE3/ASPSCR1* type 2 fusion. *GAPDH* was amplified for cDNA quality control. PCR products were separated in 3% agarose gels, extracted using the QIAquick Gel Extraction Kit (Qiagen), and sequenced by the 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The resultant PCR product sequences were compared with the human genome using BLAST from the National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

Validation of *TFE3* Break-apart Probe Set for Alveolar Soft Part Sarcoma and Xp11.2 Renal Cell Carcinoma

The histologic features of alveolar soft part sarcoma do not differ significantly between *TFE3* separation

negative, *TFE3* typical separation positive, and *TFE3* atypical separation positive cases (Figures 1a, b and c, respectively). Although Xp11.2 renal cell carcinoma with *TFE3* separation has a suggestive morphology (Figure 1d), diagnosis of these tumors can be challenging because of pathologic variation.

To identify *TFE3* rearrangements, a break-apart probe set was developed with green 3'*TFE3*, red 5'*TFE3*, and the X centromere in aqua (Figure 2a). Interphase nuclei from a normal (tumor-free) female should exhibit a 2F2A signal pattern because of an intact copy of *TFE3* on each of the two X chromosomes, whereas nuclei from a normal male should show 1F1A (Figure 3a). A typical balanced rearrangement of the *TFE3* locus would be expected to disrupt the yellow fusion signal, resulting in spatially separated green 3'*TFE3* (G) and red 5'*TFE3* (R) signals to produce a 1R1G1F2A pattern in females and a 1R1G1A pattern in males (Figure 3b). A typical unbalanced rearrangement of *TFE3* should also disrupt the fusion signal but have

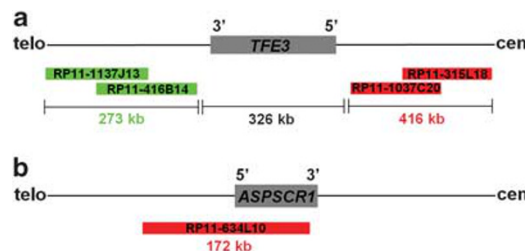


Figure 2 FISH probe design schematic. The *TFE3* break-apart probe set (a) is composed of clones flanking *TFE3* including those telomeric to 3'*TFE3* (in green), centromeric to 5'*TFE3* (in red), and a commercially available X centromere probe (in aqua, not shown). This probe set identifies disruption of the *TFE3* gene region through separation of a yellow fusion signal into red and green. The *ASPSCR1* dual-color, single-fusion probe set (b) includes clones telomeric to 3'*TFE3* (in green, shown in part a), a clone overlapping 5'*ASPSCR1* (in red), and the commercially available X centromere probe (in aqua, not shown). This probe set is a reflex test to determine if an atypical unbalanced rearrangement of *TFE3* involves fusion to *ASPSCR1*, which is identified through juxtaposition of the red and green signals to create a yellow fusion.

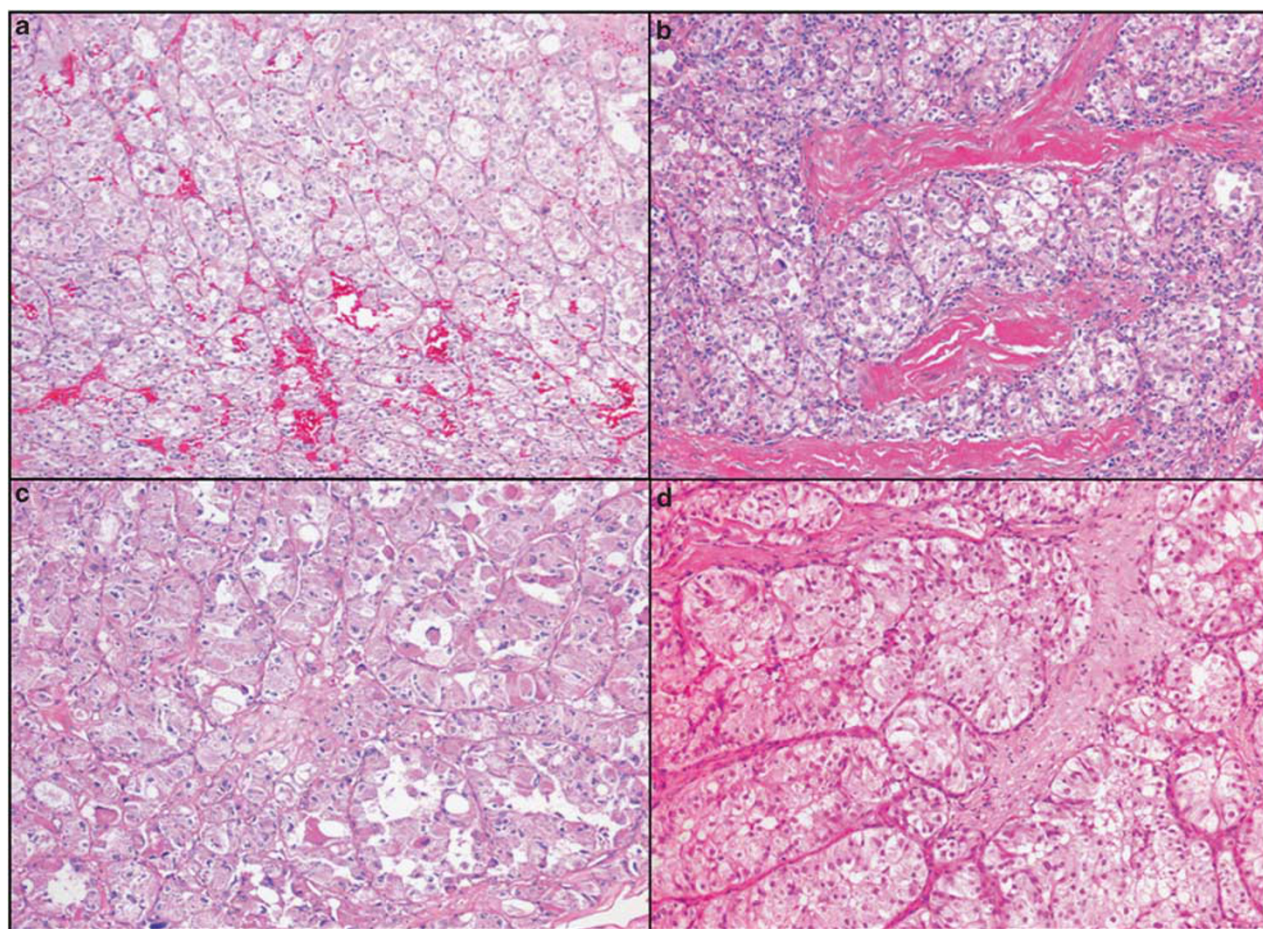


Figure 1 Histologic features of alveolar soft part sarcoma and Xp11.2 renal cell carcinoma. Alveolar soft part sarcomas are defined by large, round-to-polygonal cells containing eosinophilic granular or vacuolated cytoplasm plus one or more vesicular nuclei usually with a pseudoalveolar appearance because of the formation of distinct nests with central necrosis or loss of cohesion divided by fibrovascular septa. These features are not distinct between *TFE3* separation negative, *TFE3* typical separation positive, and *TFE3* atypical separation positive cases (a, b, and c, respectively). Xp11.2 renal cell carcinoma tumors with *TFE3* separation are heterogeneous but usually characterized by mixed papillary and nested/alveolar architecture comprised of epithelioid cells with well-defined borders containing clear and/or eosinophilic, granular, voluminous cytoplasm plus prominent nucleoli, vesicular chromatin, and psammoma bodies (d).

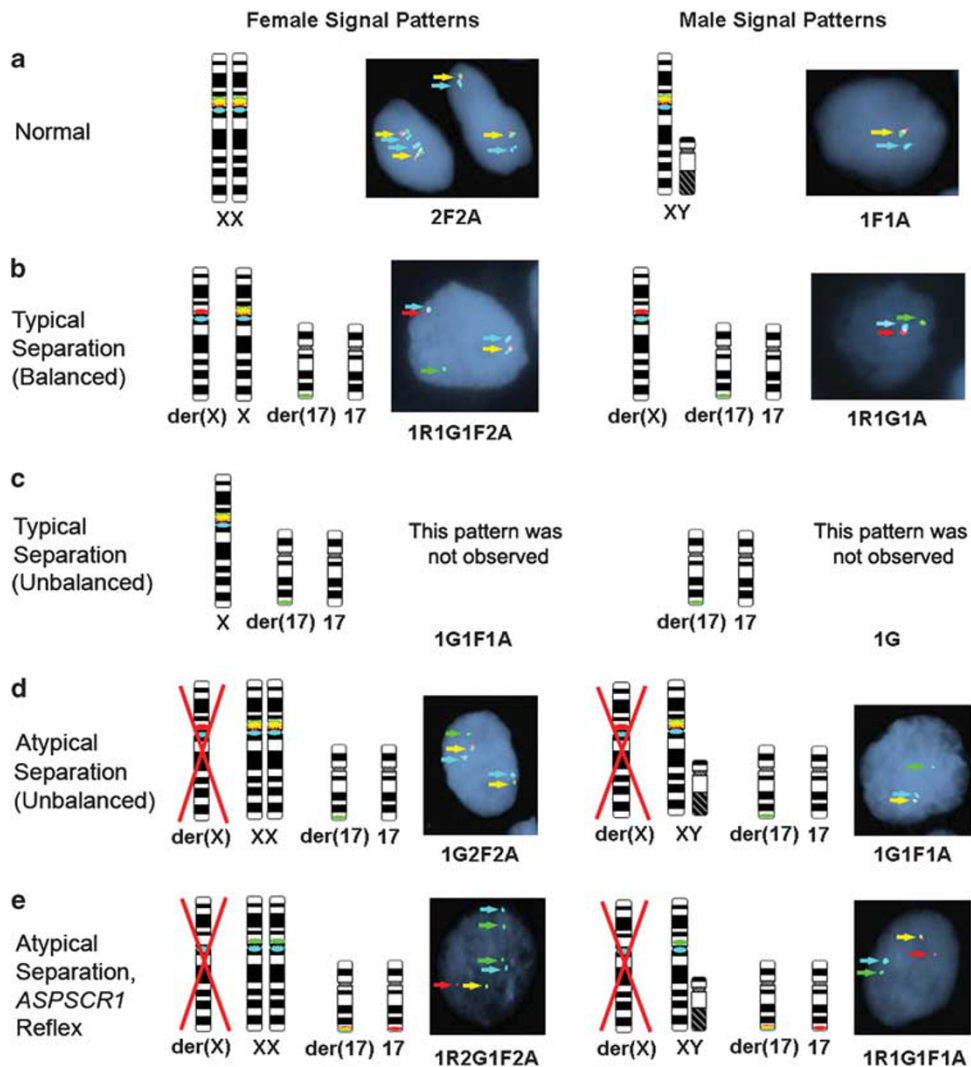


Figure 3 Probe signal patterns and interpretations for female and male cases. Normal or no separation of the *TFE3* break-apart probe set (a) is shown by yellow (fusion or F) signals with X chromosome centromere signals (aqua or A) as 2F2A and 1F1A in females and males, respectively. Typical balanced *TFE3* rearrangement (b) results in a separation of the 5' *TFE3* (red/orange or R) signal from the 3' *TFE3* (green or G) signal. The red 5' *TFE3* remains on the der(X) chromosome near the aqua X centromere, whereas the green 3' *TFE3* signal moves to the der(17). This results in a 1R1G1A pattern in males while females also have a normal X chromosome present resulting in a total signal pattern of 1R1G1F2A. A typical unbalanced rearrangement of *TFE3* (c) should also disrupt a fusion signal but with loss of the der(X) to generate a 1G1F1A pattern in females and a 1G pattern in males, although interestingly no such cases were observed. Atypical unbalanced *TFE3* rearrangement (d) represents the green 3' *TFE3* signal moving to the der(17) and loss of the der(X) plus a gain of a normal X chromosome resulting in a 1G2F2A or a 1G1F1A signal pattern in females and males, respectively. Such atypical cases are reflexed to the *ASPSCR1* dual-color, single-fusion probe set (e) to confirm 3' *TFE3* (green or G) is juxtaposed with *ASPSCR1* (red/orange or R) on the der(17) to create a yellow (fusion or F) signal, which combined with an X centromere probe (aqua or A), results in the pattern 1R2G1F2A in females and 1R1G1F1A in males.

loss of the derivative X chromosome to generate a 1G1F1A pattern in females and a 1G pattern in males (Figure 3c), although no such cases were observed; however, an atypical unbalanced separation did occur in both males and females (described in subsequent results section).

For validation, the break-apart *TFE3* probe was applied to nine alveolar soft part sarcoma, two suspected Xp11.2 renal cell carcinoma, nine differential diagnostic tumors (pheochromocytoma, metastatic melanoma, and non-Xp11.2 renal cell carcinoma), 25 non-neoplastic male control

samples, and 25 non-neoplastic female control samples (Table 1). All 50 controls were normal and the nine differential diagnostic tumors were normal or polysomic. Among the nine alveolar soft part sarcoma specimens, one was normal male (1F1A), one was polysomic for the X chromosome in a female (3F3A), one was monosomic for the X chromosome in a female (1F1A), two were typical balanced separations in females (1R1G2F2A), and four had an atypical unbalanced *TFE3* separation in two females (1G2F2A) and two males (1G1F1A). Among the two suspected Xp11.2 renal cell

Table 1 Clinicopathologic features and FISH results of validation cases

Tumor type	Gender	Age (years)	Tumor location	Size (cm)	TFE3 BAP FISH result	ASPCR1 reflex FISH result	FISH interpretation
Xp11.2 RCC-1	Female	25	Kidney	—	1G1F2A	1R1G1F2A	Atypical separation, variant ^a
Xp11.2 RCC-2	Male	67	Kidney	8 × 7 × 5.5	1F1A	—	Normal
ASPS-1	Female	— ^b	—	—	1R1G1F2A	—	Separation
ASPS-2	Female	35	Leg	17.5 × 16.5 × 15	1R1G1F2A	—	Separation
ASPS-3	Female	34	Buttock	6 × 5 × 5	1G2F2A	1R2G1F2A	Atypical separation ^c
ASPS-4	Female	37	Thigh	11 × 7.5 × 6.5	1G2F2A	1R2G1F2A	Atypical separation
ASPS-5 ^d	Male	24	Paracolic gutter	7 × 5 × 3.5	1G1F1A	1R1G1F1A	Atypical separation
ASPS-6 ^d	Male	24	Rectum	6.5 × 6 × 4.8	1G1F1A	1R1G1F1A	Atypical separation
ASPS-7	Female	33	Larynx	—	3F3A	—	Polysomy X
ASPS-8	Female	68	Stomach	5.2 × 4.2 × 3	1F1A	—	Monosomy X
ASPS-9	Male	45	Nasal mass	—	1F1A	—	Normal
Pheochromocytoma-1	Female	50	Adrenal gland	2.4 × 2.2 × 1.8	3F3A	—	Polysomy X
Pheochromocytoma-2	Female	24	Adrenal gland	5.7 × 4.2 × 1.8	3F3A	—	Polysomy X
Pheochromocytoma-3	Male	74	Adrenal gland	1.1 × 0.9 × 0.8	1F1A	—	Normal
Met Melanoma-1	Male	57	Chest wall	3 × 2.8 × 2.8	1F1A	—	Normal
Met Melanoma-2	Male	66	Ileum	5.2 × 3.5 × 1.7	1F1A	—	Normal
Met Melanoma-3	Male	59	Chest wall	—	1F1A	—	Normal
Non-Xp11.2 RCC-1	Male	62	Chest wall	1.1 × 0.9 × 0.8	1F1A	—	Normal
Non-Xp11.2 RCC-2	Male	65	Right flank	4.4 × 4.3 × 3.4	1F1A	—	Normal
Non-Xp11.2 RCC-3	Male	58	Lung	1.5 × 1.4 × 1	1F1A	—	Normal

Abbreviations: ASPS = alveolar soft part sarcoma; Met Melanoma = metastatic melanoma; RCC = renal cell carcinoma.

^aUnbalanced rearrangement with the oncogenic 3' *TFE3* on the der(17), a normal X chromosome, and a structurally abnormal X with *TFE3* loss.

^b— Denotes unknown.

^cUnbalanced rearrangement with the oncogenic 3' *TFE3* on the der(17), loss of the der(X), and gain of a normal X chromosome.

^dRepresent a primary paracolic gutter tumor and a metastatic rectal tumor from a recurrence one year later in a single individual.

carcinoma specimens, one was normal male (1F1A) and one, Case Xp11.2 RCC-1, had a variation in the atypical *TFE3* separation pattern in a female (1G1F2A), which was confirmed with the *ASPCR1* reflex probe to create a *TFE3/ASPCR1* fusion (1R1G1F2A).

Chromosome analysis of Case Xp11.2 RCC-1 demonstrated the karyotype 41-44,X,der(X)t(X;6)(p11.2;p21.3),add(1)(p13),add(3)(p11),-6,add(9)(p13),add(11)(q13),-13,add(17)(q25),-21,-22,+1-4mar[cp11]/46,XX[6], which in combination with the FISH results indicates an unbalanced *TFE3* rearrangement and suggests the oncogenic 3' *TFE3* is present on the der(17) chromosome, one normal X chromosome, and one structurally abnormal X chromosome. It is unclear whether the der(X) was completely lost as occurs in the other atypical *TFE3* separation cases because the structurally abnormal X chromosome could be derived from the der(X) or from a normal X chromosome, which then subsequently had a portion of the p-arm encompassing the *TFE3* region moved to chromosome 6 followed by loss of the der(6).

Atypical Separation Due to an Unbalanced Rearrangement with an Additional Normal Chromosome X

A dual-color, single-fusion strategy reflex probe set with green 3' *TFE3*, red 5' *ASPCR1*, and aqua X centromere was developed to further investigate the etiology of the atypical separation pattern

(Figure 2b). Interphase nuclei from a normal female would be expected to have a 2R2G2A signal pattern, whereas nuclei from a normal male should demonstrate 1R1G1A. Fusion of *TFE3/ASPCR1* is denoted by a single yellow signal that is created through juxtaposition of the red and green probes. In addition, although not observed during the validation, this reflex probe set can confirm a typical unbalanced rearrangement in males represented by a signal pattern of 1G using break-apart *TFE3* (retention of der(17) but loss of der(X)); such confirmation is important because a single signal is difficult to differentiate from tissue cut artifact.

The atypical separation patterns that were observed during validation with break-apart *TFE3* (1G2F2A or 1G1F1A) were determined using the *ASPCR1* reflex probe set to result from an unbalanced translocation with retention of the der(17) and loss of the der(X) as well as the unexpected gain of an additional normal X chromosome in both males and females. Specifically, in four such validation cases of ASPS, the *ASPCR1* reflex probe set demonstrated a 1R2G1F2A pattern in the two females and a 1R1G1F1A pattern in the two males. The signal patterns for these atypical results for both the break-apart *TFE3* and reflex *ASPCR1* probe sets are further explained (Figures 3d and e). In addition, these atypical pattern findings were consistent with sequential metaphase FISH using both probe sets (Figures 4b and c) on an alveolar soft part sarcoma sample from a female with an unbalanced rearrangement identified through karyotype review (Figure 4a). Another alveolar soft part

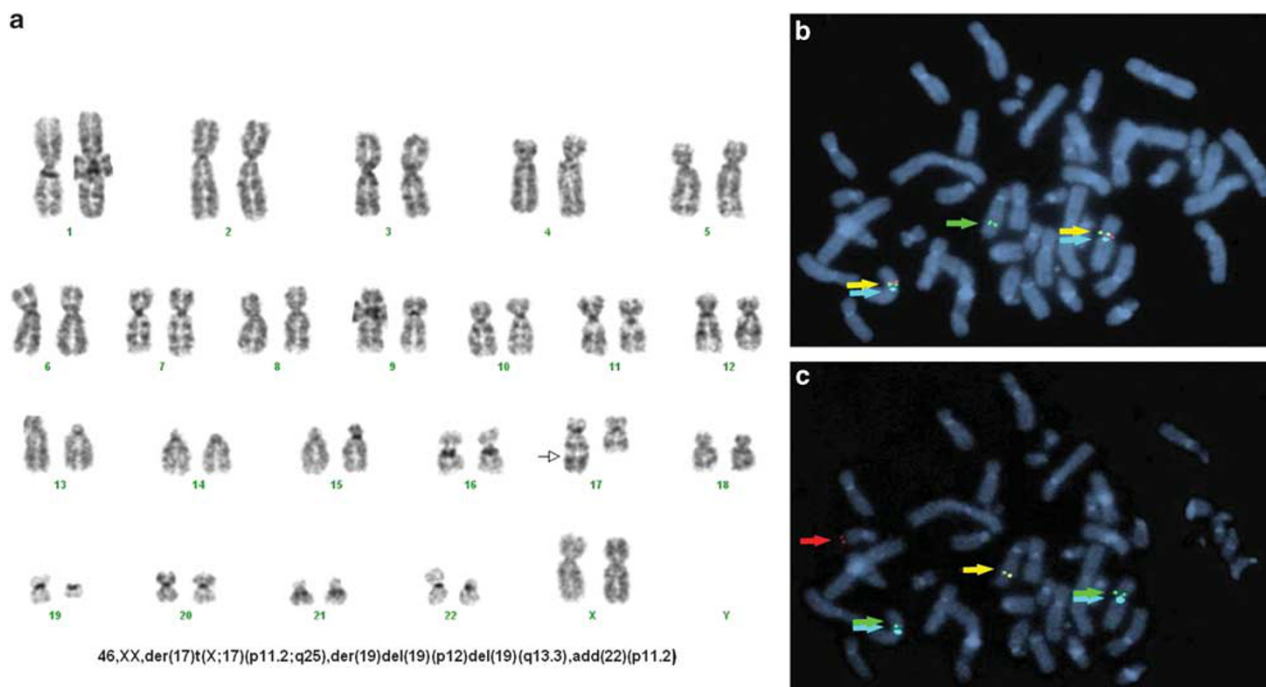


Figure 4 Analysis of an alveolar soft part sarcoma case retrospectively collected based on karyotype clarified the cause of the atypical fusion pattern observed by FISH is an unbalanced X;17 translocation with gain of a normal X chromosome. The karyotype (a) showed a der(17) chromosome without the reciprocal der(X) plus two normal X chromosomes in this female patient. The *TFE3* break-apart probe set (b) produced a 1G2F2A signal pattern representing one green 3'*TFE3* signal on the der(17) and two normal X chromosomes, each with one intact *TFE3* yellow fusion signal and one aqua X centromere signal. Confirmation with the reflex *ASPSCR1* probe set (c) produced a 1R2G1F2A pattern due to two normal X chromosomes that each have one green 3'*TFE3* signal and one aqua X centromere signal, a single normal chromosome 17 with a red 5'*ASPSCR1* signal, and one yellow 3'*TFE3*/5'*ASPSCR1* fusion signal on the der(17) chromosome.

sarcoma case in a male with an atypical unbalanced separation signal pattern by FISH (Figure 5a) was confirmed to have *TFE3/ASPSCR1* fusion by RT-PCR and sequencing of the resulting cDNA (Figure 5b).

Finally, to investigate the etiology of the atypical unbalanced separation pattern specifically in renal cell carcinoma, as no karyotype results were available on any renal cell carcinoma validation sample, an additional female case of renal cell carcinoma with a complex Xp11.2 translocation identified by chromosome analysis was ascertained through a retrospective search (Figure 6a). Sequential metaphase FISH with the break-apart *TFE3* probe set resulted in confirmation of *TFE3* separation (Figure 6b) and suggested a potential mechanism for this complex rearrangement (Figure 6c). This unique alteration has not previously been described and represents a divergence from reported cases where the *TFE3/ASPSCR1* oncogenic fusion is characteristically located on the der(17) chromosome.

Inv(X) Variant of Xp11.2 Renal Cell Carcinoma

One of the recurrent Xp11.2 rearrangement variants in renal cell carcinoma is an inv(X)(p11.2q12) that is known to fuse *TFE3* and *NONO*. The associated

subtle shift of chromatin near the centromere is demonstrated by an ideogram and a metaphase image of chromosome X, which was obtained from analysis of the UOK-109 cell line that is known to carry this pericentric inversion (Figure 7a). The break-apart *TFE3* FISH probe set was confirmed to detect the inv(X) by both metaphase (Figure 7b) and interphase (Figure 7c) analyses on the UOK-109 cell line.

Clinical Experience

Within the first 14 months of clinically offering the *TFE3* FISH test, 75 cases have been studied (Table 2). Among the suspected renal cell carcinoma cases ($n = 54$), 14 had a typical balanced *TFE3* separation (cases 1–14), 23 were normal (cases 32–54), seven had at least one additional normal X chromosome without *TFE3* separation (polysomy, cases 20–26), whereas four females had monosomy X (cases 28–31). One case without *TFE3* separation in a male also had cells with an additional normal X chromosome and cells with an additional normal Y chromosome (polysomy for both X and Y, case 27). Further, five cases had balanced *TFE3* separation with a pattern suggestive of the opposite gender than expected, which was confirmed by FISH with probes for the centromeres of chromosomes X and Y.

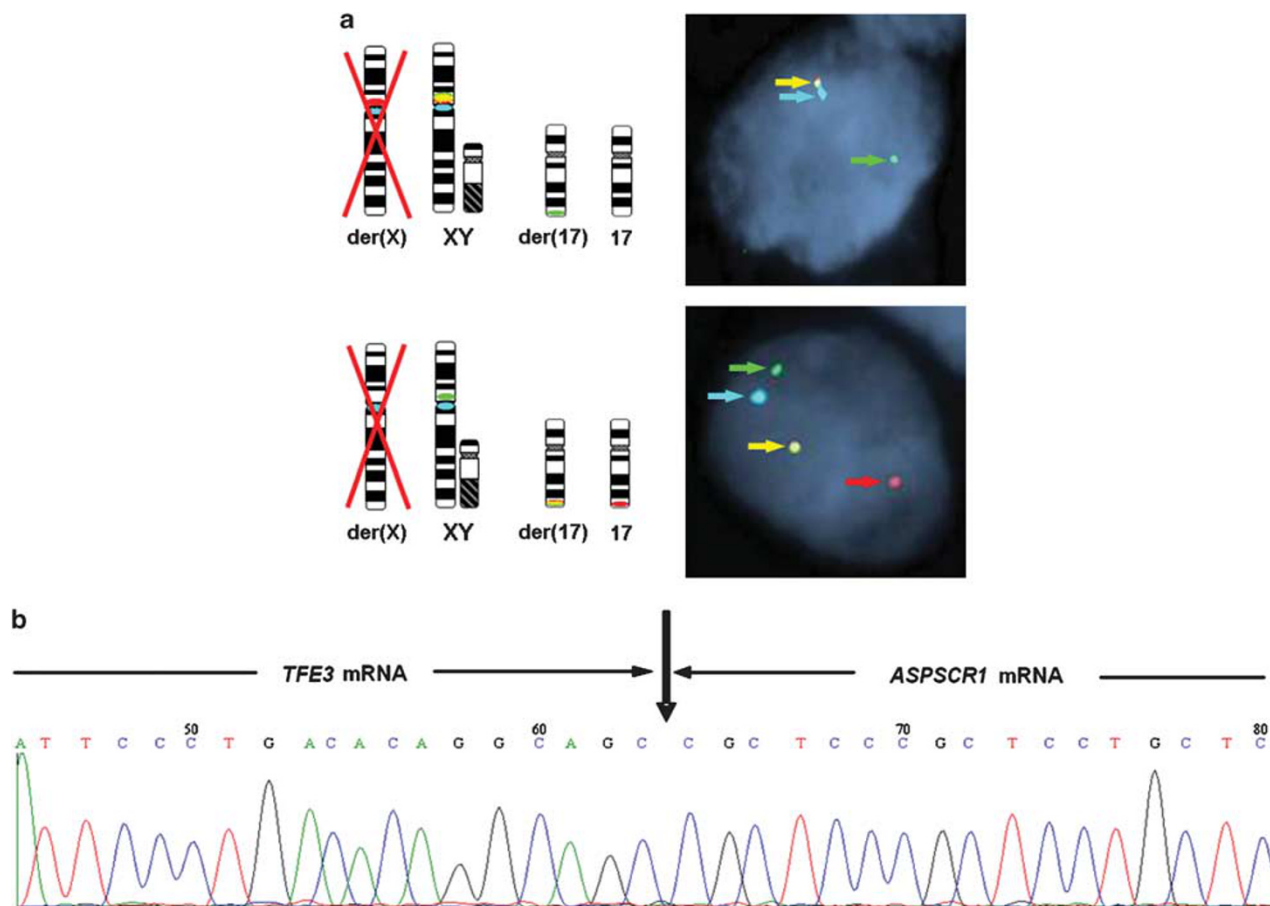


Figure 5 Sequencing confirmed *TFE3/ASPSCR1* fusion occurs when the atypical unbalanced separation signal pattern is observed by FISH. In male patient ASPS-5, the atypical separation pattern by interphase FISH with the *TFE3* break-apart probe set is 1G1F1A and with the reflex *ASPSCR1* probe set is 1R1G1F1A (a). RT-PCR and sequencing of the resultant cDNA showed fusion between *TFE3* and *ASPSCR1* (b).

Referring physicians verified the initially provided patient genders were correct. Two of these cases were males with gain of an additional normal chromosome X and loss of the Y chromosome within the *TFE3* separation cells as well as a population of normal male cells (cases 17 and 18). One of the male cases had a typical *TFE3* separation pattern for a male plus a cell line with two normal X chromosomes and a Y chromosome suggesting polysomy X (case 19). Finally, two of these cases were females with a cell line showing *TFE3* separation with loss of one normal X chromosome as well as a monosomy X cell line; both abnormal cell lines did not have a chromosome Y present and a minor population of normal female cells was also noted (cases 15 and 16).

Among the suspected alveolar soft part sarcoma cases ($n=13$), three had a typical balanced *TFE3* separation (cases 55–57), four were normal (cases 64–67), and one male had a single additional normal X chromosome without *TFE3* separation (polysomy, case 63). In addition, five had an atypical unbalanced separation (three females and two males) as was observed in the validation study, which reflex

testing with the *ASPSCR1* probe set confirmed resulted in *TFE3/ASPSCR1* fusion (cases 58–62). A small number of other samples were also tested including two cases of suspected PEComa and one case of chordoma, which were normal as well as cases with a nonspecific reason for referral ($n=5$).

Of the clinical cases found to be positive by *TFE3* FISH, review of all available clinical findings, morphology, and IHC confirmed concordance of the diagnostic impressions with the expectation of *TFE3* rearrangement (ie, a likely diagnosis of alveolar soft part sarcoma or Xp11.2 renal cell carcinoma).

Discussion

In recent years, the genetic signatures of multiple tumor types have been defined by rearrangement of *TFE3* at chromosome Xp11.2. To aid in diagnosis and prognosis of these entities, a break-apart strategy FISH probe set for *TFE3* with an X centromere control and a reflex *TFE3/ASPSCR1*

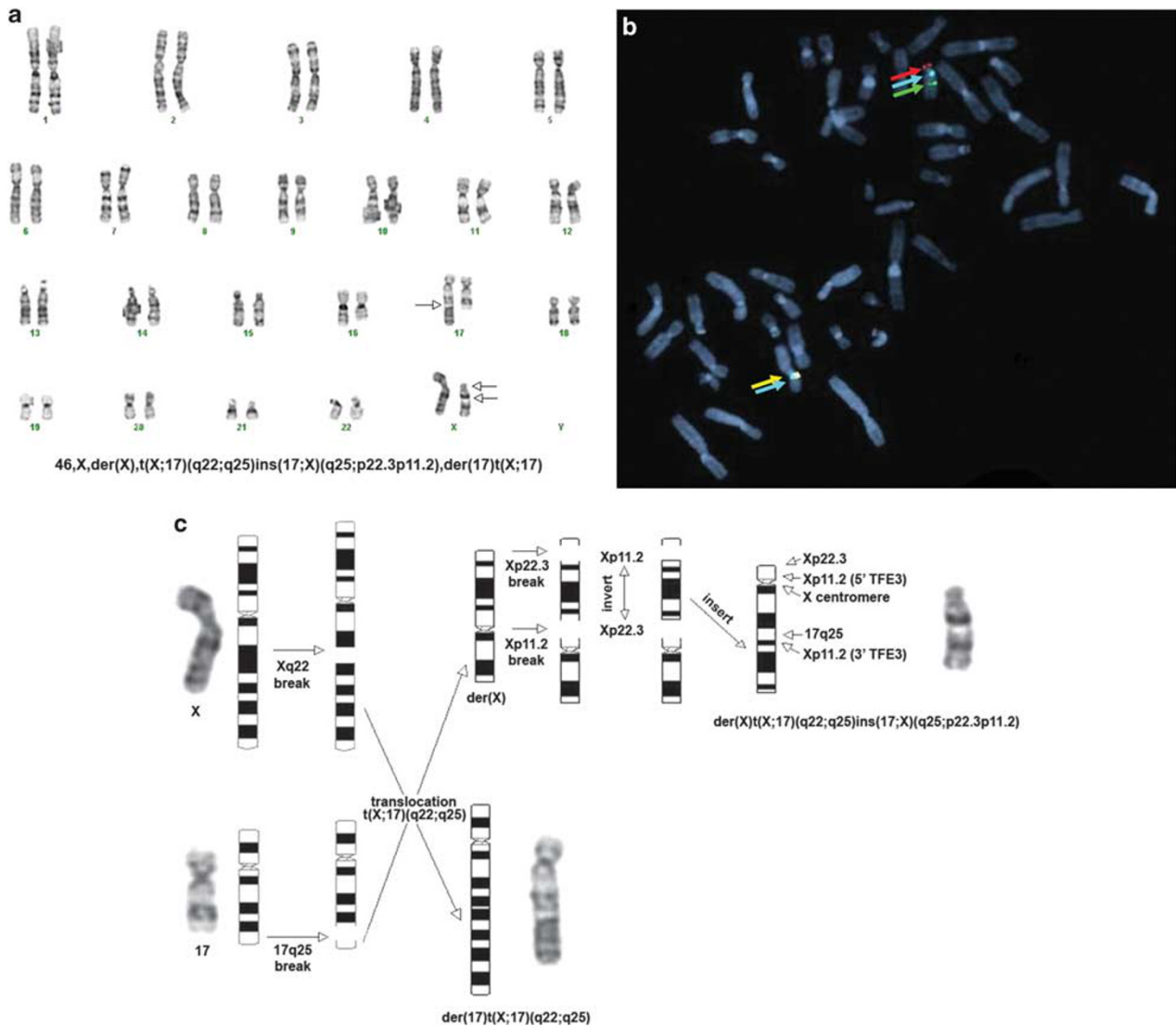


Figure 6 Analysis of a renal cell carcinoma case retrospectively collected based on karyotype demonstrated the *TFE3* break-apart probe can clarify complex rearrangements in Xp11.2 renal cell carcinoma. In this female patient, a potential mechanism based on the chromosome (a) and FISH (b) patterns is a balanced translocation between one chromosome Xq22 and chromosome 17q25 as the initial event (c). The der(X) then had breaks at bands p22.3 and p11.2. The segment in between the breaks inverted and was inserted back into the der(X) within the chromosome 17q25 portion that had been attached during the initial translocation. This brought the 3' *TFE3* at Xp11.2 and 5' *ASPSCR1* at 17q25 into proximity to form the oncogenic fusion. Such a result is unique as all previously described cases of *TFE3/ASPSCR1* had the oncogenic fusion on the der(17) chromosome. The final karyotype is 46,X,der(X)t(X;17)(q22;q25)ins(17;X)(q25;p22.3p11.2),der(17)t(X;17).

dual-color, single-fusion strategy FISH probe set were developed, validated, and then applied to 75 clinical cases of suspected Xp11.2 renal cell carcinoma, alveolar soft part sarcoma, or PEComas. Importantly, the validation included examination of break-apart *TFE3* on the UOK-109 renal carcinoma cell line, confirming detection by both metaphase and interphase FISH of the recurrent but very subtle inversion near the centromere of chromosome X, which creates *TFE3/NONO* fusion.²¹ Neither the ability to identify this subtle inversion nor use of an X centromere control probe and *ASPSCR1* reflex probe set were described in a previous publication of a *TFE3* break-apart probe set.³⁰ Another study did

utilize the UOK-109 cell line for validation, although the other controls were not employed.³⁸ In addition, a recent abstract describes the clinical experience in a renal consultation service with application of a *TFE3* break-apart FISH assay to 62 cases with 21 positive results, although details about the probe design, validation, and resulting signal patterns were not included.³² The current study is therefore, to the best of our knowledge, the first to address the potential for an unbalanced *TFE3* rearrangement in a male resulting in only a single signal, which is challenging to distinguish from tissue cut artifact but can be resolved using our reflex *ASPSCR1* probe set.

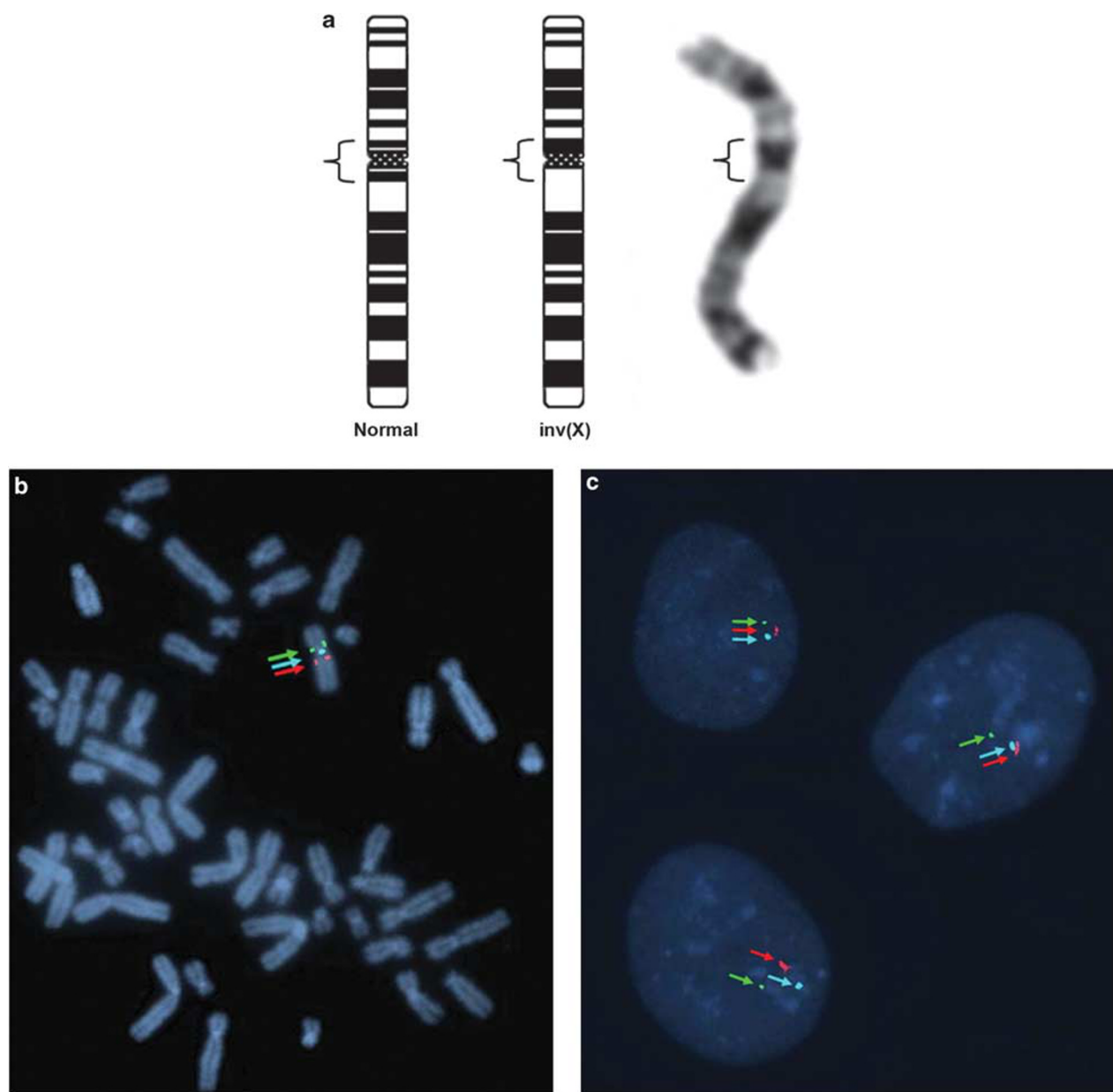


Figure 7 The Xp11.2 renal cell carcinoma-associated pericentric inversion of X chromosome can be detected by the *TFE3* break-apart FISH probe. The UOK-109 cell line carries a single X chromosome that has inv(X)(p11.2q12). This creates a *TFE3/NONO* fusion and is a subtle movement of chromatin near the centromere from one chromosome arm to the other (a). FISH with the *TFE3* break-apart probe set on the UOK-109 cell line showed a 1R1G1A pattern consistent with *TFE3* rearrangement on the X chromosome by both metaphase and interphase analysis (b and c, respectively).

It was unexpected that a typical unbalanced *TFE3* rearrangement with simple loss of the der(X) was not detected in any case. Instead, the break-apart *TFE3* probe set pattern in some cases was 1G2F2A in females and 1G1F1A in males, indicating the presence of 3'*TFE3* on the oncogenic der(17) with loss of the der(X) but also surprisingly suggesting gain of a normal X chromosome. The *ASPSCR1* reflex probe set confirmed that 3'*TFE3* fused to *ASPSCR1* at 17q25.3 in these atypical unbalanced cases. Karyotype confirmation of these probe patterns was acquired by a retrospective case search

that identified an alveolar soft part sarcoma karyotype including two normal X chromosomes and a der(17)t(X;17)(p11.2;q25). Rare karyotypes demonstrating a similar der(17) and an additional normal X chromosome have been reported previously in both male and female patients.^{17,39–42} Such an atypical unbalanced separation pattern was further noted in five of the cases tested clinically. Other recurrent sex chromosome copy number combinations involving both gain and loss were also observed.

Historically, translocation of chromosomes X and 17 resulting in *TFE3/ASPSCR1* fusion was reported

Table 2 Clinicopathologic features and FISH results of clinical cases

Case	Gender	Age (years)	Tumor location	Size (cm)	Working diagnosis	TFE3 BAP FISH result	ASPSR1 reflex FISH result	XY probe for sex determination ^a	FISH interpretation
1	Female	30	Kidney	— ^b	Renal cell carcinoma	1R1G1F2A	—	—	Separation
2	Female	42	Kidney	—	Renal cell carcinoma	1R1G1F2A	—	—	Separation
3	Female	40	Kidney	14.8 × 12.6 × 11.5	Renal cell carcinoma	1R1G1F2A	—	—	Separation
4	Female	26	Kidney	1.5	Renal cell carcinoma	1R1G1F2A	—	—	Separation
5	Female	33	Kidney	—	Renal cell carcinoma	1R1G1F2A	—	—	Separation
6	Female	40	Kidney	3.8 × 3.1 × 3.0	Renal cell carcinoma	1R1G1F2A	—	—	Separation
7	Female	29	Kidney	4.5 × 4.5 × 5.6	Renal cell carcinoma	1R1G1F2A	—	—	Separation
8	Female	18	Kidney	4.5 × 3.7 × 1.4	Renal cell carcinoma	1R1G1F2A	—	—	Separation
9	Female	34	Kidney	16 × 13 × 11.5	Renal cell carcinoma	1R1G1F2A	—	—	Separation
10	Female	47	Kidney	1.7 × 1.3 × 0.5	Renal cell carcinoma	1R1G1F2A	—	—	Separation
11	Male	35	Kidney	—	Renal cell carcinoma	1R1G1A	—	—	Separation
12	Male	3	Kidney	15 × 11 × 8	Renal cell carcinoma	1R1G1A	—	—	Separation
13	Male	56	Kidney	6 × 4.5 × 2.5	Renal cell carcinoma	1R1G1A	—	—	Separation
14	Male	48	Kidney	—	Renal cell carcinoma	1R1G1A	—	—	Separation
15	Female	59	Kidney	11 × 7 × 4.5	Renal cell carcinoma	1R1G1A/1F1A ^c	—	1G/2G	Separation (male)/X (monosomy X)/XX (normal female)
16	Female	34	Kidney	4.6 × 3.5 × 3.3	Renal cell carcinoma	1R1G1A/1F1A ^c	—	1G/2G	Separation (male)/X (monosomy X)/XX (normal female)
17	Male	46	Kidney	21 × 13 × 9	Renal cell carcinoma	1R1G1F2A/1F1A	—	2G/1R1G	Separation (female)/XY (normal male)
18	Male	71	Kidney	1.5 × 1.5 × 0.9	Renal cell carcinoma	1R1G1F2A/1F1A	—	2G/1R1G	Separation (female)/XY (normal male)
19	Male	35	Kidney	9.1 × 6.5 × 2.2	Renal cell carcinoma	1R1G1A/2F2A	—	1R1G/1R2G	Separation (male)/XXY (polysomy X)
20	Female	50	Kidney	—	Renal cell carcinoma	3F3A	—	—	Polysomy X
21	Female	50	Kidney	—	Renal cell carcinoma	3F3A	—	—	Polysomy X
22	Female	45	Kidney	—	Renal cell carcinoma	3-4F3-4A	—	—	Polysomy X
23	Female	13	Kidney	—	Renal cell carcinoma	3-4F3-4A	—	—	Polysomy X
24	Female	85	Kidney	—	Renal cell carcinoma	3-6F3-6A	—	—	Polysomy X
25	Female	24	Kidney	7.2 × 6.1 × 4	Renal cell carcinoma	3F3A	—	—	Polysomy X
26	Male	31	Groin	—	Renal cell carcinoma	2F2A	—	—	Polysomy X
27	Male	30	Kidney	15 × 9 × 7	Renal cell carcinoma	2F2A/1F1A	—	2G1R/ 1G2R/1G1R	XXY (polysomy X)/XYY (polysomy Y)/XY (normal male)
28	Female	49	Kidney	—	Renal cell carcinoma	1F1A	—	—	Monosomy X
29	Female	49	Kidney	—	Renal cell carcinoma	1F1A	—	—	Monosomy X
30	Female	55	Kidney	—	Renal cell carcinoma	1F1A	—	—	Monosomy X
31	Female	42	Kidney	—	Renal cell carcinoma	1F1A	—	—	Monosomy X
32	Female	68	Kidney	11.2 × 10.7 × 9.5	Renal cell carcinoma	2F2A	—	—	Normal
33	Female	54	Kidney	2.7	Renal cell carcinoma	2F2A	—	—	Normal
34	Female	19	Kidney	—	Renal cell carcinoma	2F2A	—	—	Normal
35	Female	26	Kidney	—	Renal cell carcinoma	2F2A	—	—	Normal
36	Female	65	Kidney	4.8 × 4.4	Renal cell carcinoma	2F2A	—	—	Normal
37	Female	29	Kidney	6 × 4.5 × 1	Renal cell carcinoma	2F2A	—	—	Normal
38	Female	74	Kidney	0.6 × 1.4 × 0.1	Renal cell carcinoma	2F2A	—	—	Normal
39	Female	34	Parotid Mass	8.3 × 6.2 × 3.7	Renal cell carcinoma	2F2A	—	—	Normal
40	Female	23	Kidney	8 × 5 × 4	Renal cell carcinoma	2F2A	—	—	Normal
41	Male	56	Interaortocaval lymph node	—	Renal cell carcinoma	1F1A	—	—	Normal
42	Male	47	Kidney	3 × 2.6 × 2.5	Renal cell carcinoma	1F1A	—	—	Normal
43	Male	64	Kidney	4.6 × 4.5 × 4.2	Renal cell carcinoma	1F1A	—	—	Normal
44	Male	20	Liver	—	Renal cell carcinoma	1F1A	—	—	Normal
45	Male	51	Kidney	12 × 11 × 8.4	Renal cell carcinoma	1F1A	—	—	Normal
46	Male	27	Supraclavicular lymph node	3 × 2.2 × 1.2	Renal cell carcinoma	1F1A	—	—	Normal
47	Male	41	Kidney	2.5 × 2.5 × 2	Renal cell carcinoma	1F1A	—	—	Normal
48	Male	60	Kidney	17 × 10 × 9.3	Renal cell carcinoma	1F1A	—	—	Normal
49	Male	65	Kidney	15 × 9 × 9	Renal cell carcinoma	1F1A	—	—	Normal
50	Male	60	Kidney	4.2 × 3.5 × 3.5	Renal cell carcinoma	1F1A	—	—	Normal
51	Male	16	Kidney	3.3 × 2.5 × 1.6	Renal cell carcinoma	1F1A	—	—	Normal
52	Male	65	Kidney	—	Renal cell carcinoma	1F1A	—	—	Normal
53	Male	45	Kidney	11.5 × 9 × 8.6	Renal cell carcinoma	1F1A	—	—	Normal
54	Male	68	Kidney	13 × 11 × 8	Renal cell carcinoma	1F1A	—	—	Normal
55	Female	30	Popliteal fossa	—	Alveolar soft part sarcoma	1R1G1F2A	—	—	Separation
56	Female	19	Vagina	0.8 × 0.8 × 0.2	Alveolar soft part sarcoma	1R1G1F2A	—	—	Separation
57	Male	25	Lung	0.9 × 0.4 × 0.4	Alveolar soft part sarcoma	1R1G1A	—	—	Separation
58	Female	37	Left popliteal fossa	0.5 × 0.5 × 0.1	Alveolar soft part sarcoma	1G2F2A	1R2G1F2A	—	Atypical separation ^d
59	Female	38	Buttock	9 × 8.5 × 6.0	Alveolar soft part sarcoma	1G2F2A	1R2G1F2A	—	Atypical separation

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TFE3 FISH in Xp11.2 renal cell carcinoma

Table 2 (Continued)

Case	Gender	Age (years)	Tumor location	Size (cm)	Working diagnosis	TFE3 BAP FISH result	ASPCR1 reflex FISH result	XY probe for sex determination ^a	FISH interpretation
60	Female	48	Rib bone ^b	—	Alveolar soft part sarcoma	1G2F2A	1R2G1F2A	—	Atypical separation
61	Male	30	Thigh	—	Alveolar soft part sarcoma	1G1F1A	1R1G1F1A	—	Atypical separation
62	Male	39	Right posterior neck	7.1 × 4.7 × 3.3	Alveolar soft part sarcoma	1G1F1A	1R1G1F1A	—	Atypical separation
63	Male	48	Supraglottic mass	2 × 1 × 0.3	Alveolar soft part sarcoma	2F2A	—	—	Polysony X
64	Female	43	Left sinus	—	Alveolar soft part sarcoma	2F2A	—	—	Normal
65	Male	31	Thigh	—	Alveolar soft part sarcoma	1F1A	—	—	Normal
66	Male	7	Thigh	—	Alveolar soft part sarcoma	1F1A	—	—	Normal
67	Male	71	Portohepatis lymph node	1.7 × 0.3 × 0.1	Alveolar soft part sarcoma	1F1A	—	—	Normal
68	Female	16	Ovary	—	Alveolar soft part sarcoma	1R1G1F2A	—	—	Separation
69	Female	72	Thoracic vertebra T12	3.5 × 2.5 × 0.6	—	3F3A	—	—	Polysony X
70	Male	86	Skull	—	—	2F2A	—	—	Polysony X
71	Female	48	Ovary and fallopian tube ^c	8.5 × 8.0 × 6.5	—	1F1A	—	—	Monosomy X
72	Female	42	—	—	—	1F1A	—	—	Monosomy X
73	Male	52	Presacral mass	—	Chordoma	1F1A	—	—	Normal
74	Male	10	Brain	—	PEComa	1F1A	—	—	Normal
75	Male	42	Thigh	—	PEComa	1F1A	—	—	Normal

^aChromosome X centromere probe (DXZ1 in green) and chromosome Y centromere probe (DYZ3 in red).

^b— Denotes unknown.

^cApproximately 10% of cells had a 2F2A pattern (normal cells in this female patient); this is lower than expected relative to the XY centromere probe results as TFE3 BAP scoring was biased toward the abnormal signal patterns.

^dUnbalanced rearrangement with the oncogenic 3' TFE3 on the der(17), loss of the der(X), and gain of a normal X chromosome.

^eRepresents an ovary/fallopian tube tumor and a rib bone tumor that may be unrelated malignancies in a single individual.

as being present in a balanced form in Xp11.2 renal cell carcinoma and in an unbalanced form with loss of the derivative chromosome X in alveolar soft part sarcoma. However, our combined validation and clinical sample results demonstrate unbalanced TFE3 rearrangements in one Xp11.2 renal cell carcinoma and nine alveolar soft part sarcoma cases as well as balanced TFE3 rearrangements in 19 Xp11.2 renal cell carcinoma and five alveolar soft part sarcoma cases. This is consistent with 18 alveolar soft part sarcoma tumors with TFE3/ASPCR1 fusion that were shown by RT-PCR to be balanced in four cases and unbalanced in the remainder.³⁷ Other alveolar soft part sarcoma cases with a balanced form of the TFE3 rearrangement have been reported.^{17,43} These results suggest the balanced versus unbalanced nature of the translocation as being based on tumor type is a misconception likely due to the limited numbers of initial cases or to a preference but not a necessity of the different tumors.

The new break-apart TFE3 probe set was also recently used to identify an approximately 1% frequency of TFE3 rearrangement in 632 consecutively treated adult renal cell carcinoma patients, with one of the six positive tumors also demonstrating TFE3/ASPCR1 fusion using the ASPSCR1 reflex probe set.⁸ Two of the abnormal TFE3 break-apart probe results had a 2R2G pattern in males, suggesting rearrangement of two TFE3 copies, which was not a pattern seen during the probe validation or subsequent clinical experience. Of importance, significantly worse cancer-specific survival was found for Xp11.2 papillary-type renal cell carcinoma compared with TFE3 negative papillary renal cell carcinoma.⁸

This result is consistent with other literature suggesting Xp11.2 renal cell carcinoma has a relatively more aggressive clinical course in adults.^{3,9-14} For example, in one of the largest reports to date, half of the 28 adults (≥20 years of age at diagnosis) with Xp11.2 renal cell carcinoma presented at stage IV and 85% of cases in which lymph nodes were resected were positive for metastasis.⁹ In another study, five Xp11.2 adult renal cell carcinoma cases had a poor clinical course with a mean survival of 18 months post diagnosis.¹⁴ Analysis of 54 Xp11.2 renal cell carcinoma cases also demonstrated that the presence of distant metastases was the only clinicopathologic difference between patients younger or older than 25 years of age, indicating that age and lymph node involvement are prognostic factors for recurrence of TFE3-rearranged renal cell carcinoma.¹³ Finally, adults not only often present with widespread systemic metastases and a poor prognosis, Xp11.2 renal cell carcinoma tumors diagnosed at any age also have the potential to metastasize decades after the initial presentation.^{3,6,44,45}

This apparent aggressive course in adults is important despite the rarity of Xp11.2 renal cell carcinoma in this population relative to the approximately 40% of pediatric cases.^{3,4} As adults have a

much higher overall renal cell carcinoma incidence of approximately 25 000 cases per year in the United States,⁴⁶ the majority of Xp11.2 renal cell carcinoma cases will likely occur in the adult population. This frequency and the growing literature supporting a negative prognostic significance for *TFE3* rearrangement in adult renal cell carcinoma suggests screening may be important to identify this variant in older individuals.

In the pediatric population, Xp11.2 renal cell carcinoma is generally associated with an advanced stage at presentation (ie, regional nodal metastasis without hematogenous spread) but has a favorable short-term prognosis. This is highlighted in a literature review which showed >90% of patients remained disease free at last follow-up with a mean of 6.3 years.⁷ It is of note though that there is a paucity of long-term follow-up information. As such a favorable prognosis remains true after surgery independently of adjuvant therapy, this led to the suggestion that young renal cell carcinoma patients should also be routinely screened for *TFE3* rearrangement to help determine the appropriate use of adjuvant therapy.⁷

Screening only by histology is not suitable to diagnose Xp11.2 renal cell carcinoma because of morphologic features that can overlap with both clear cell and papillary renal cell carcinoma. Xp11.2 renal cell carcinoma has papillary and/or nested architecture with cells containing clear, voluminous, and/or eosinophilic cytoplasm plus few to extensive psammoma bodies. Such a morphologically heterogeneous entity can be mistaken for rare clear cell renal cell carcinoma with a focal papillary architecture or with pseudopapillary areas arising from degeneration of acinar structures.^{4,6,9,14,47} Xp11.2 renal cell carcinoma can also be misdiagnosed as papillary renal cell carcinoma when cytoplasmic clearing is present because of degeneration associated with hemosiderin deposition as well as when the Xp11.2 renal cell carcinoma has prominent eosinophilic cytoplasm that can mimic type 2 papillary renal cell carcinoma.^{3,4,6,9,14}

IHC is a potential screening tool for Xp11.2 renal cell carcinoma but does not have sufficient sensitivity or specificity to establish *TFE3* rearrangement; true *TFE3* disruption was confirmed by FISH only in four of the nine (44%) well-characterized renal cell carcinoma tumors that had moderate-to-strong nuclear TFE3 immunoreactivity in each of two recent studies,^{28,29} and fixation-related artifacts have been reported, such as an affinity for the edges as well as variability in antibody batch performance.^{30,48,49} Similar findings occurred for alveolar soft part sarcoma where only 22 of the 24 cases of *TFE3/ASPSCR1* confirmed by FISH were positive by IHC and, importantly, *TFE3* immunoreactivity was also noted in two of five non-alveolar soft part sarcoma neoplasms (granular cell tumors) that were negative by FISH.³¹ Therefore, the diagnosis of Xp11.2 renal cell carcinoma and alveolar soft part

sarcoma requires genetic confirmation using a method such as FISH or RT-PCR to supplement histology and IHC results in diagnostically challenging cases. Owing to reports of Xp11.2 rearrangement in renal cell carcinoma with currently unknown partners,^{9,34} and likely future discoveries of additional fusion variants, FISH for *TFE3* rearrangement is the preferred method to RT-PCR as it does not require knowledge of the partner gene and is a highly sensitive method without the technical challenge of RNA degradation in formalin-fixed, paraffin-embedded tissue samples.

Interestingly, Xp11.2 renal cell carcinoma has shown similar behavior to that of alveolar soft part sarcoma as several reports demonstrate cases of delayed recurrence and chemotherapy resistance.^{3,6,44,45} Alveolar soft part sarcoma and renal cell carcinoma can further overlap in morphology with nested and alveolar growth patterns bounded by prominent sinusoidal vasculature with polygonal cells containing clear to eosinophilic cytoplasm and distinct borders.^{25,50–52} In addition, gene expression studies suggest Xp11.2 renal cell carcinoma is more closely related to alveolar soft part sarcoma than to typical clear cell renal cell carcinoma.⁵³ These studies suggest that Xp11.2 renal cell carcinoma may require a different treatment strategy without adjuvant chemotherapy compared with *TFE3*-negative renal cell carcinoma.

In alveolar soft part sarcoma, rearrangement of *TFE3* with *ASPSCR1* has also been reported.¹⁷ Diagnosis of these tumors is typically straightforward but can be challenging when presenting as a metastasis, in an unusual location, or without typical histologic features. Alveolar soft part sarcoma can mimic multiple other neoplasms, particularly those that show nested or organoid patterns of growth with cells containing abundant eosinophilic cytoplasm (such as granular cell tumor, paraganglioma, clear cell sarcoma, and metastatic clear cell renal cell carcinoma), which have markedly different management strategies.²⁷

In addition to alveolar soft part sarcoma and Xp11.2 renal cell carcinoma, rearrangements of *TFE3* have also recently been identified in PEComas; *TFE3* expression was shown using IHC in five of 17 cases and fusion of *PSF/TFE3* was established in one case by RT-PCR and FISH.^{54,55} Subsequently, four extra-renal site *TFE3*-rearranged PEComas were identified that suggested an association with younger age, lack of tuberous sclerosis, and prominent epithelioid cells with alveolar architecture, although fusion with *PSF* was not detected in the two cases tested.³³

In summary, we have validated a FISH probe set for use in formalin-fixed, paraffin-embedded tissues to detect *TFE3* rearrangement with a reflex strategy to identify *TFE3/ASPSCR1* fusion. Use of these probes in clinical practice on a large number of cases has demonstrated their value as a diagnostic aid and provided an important resource to understand the

complexity of *TFE3* interpretation. This FISH assay is a powerful and necessary tool to identify Xp11.2 renal cell carcinoma as distinct from clear and papillary renal cell carcinoma, alveolar soft part sarcoma as a metastasis or with unusual morphology, and PEComas. The importance of a robust FISH assay will have a key role as the understanding of the prognostic significance of *TFE3* rearrangement in Xp11.2 renal cell carcinoma unfolds and other tumors are defined by alteration of this gene.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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