# Renal Cell Carcinoma Associated with Xp11.2 Translocation/TFE3 Gene Fusions: Clinical Features, Treatments and Prognosis

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## Abstract

To investigate the clinical characteristics, treatments and prognosis of renal cell carcinoma associated with Xp11.2 translocation/TFE3 gene fusions (Xp11.2 tRCC), the epidemiological features and treatment results of 34 cases of Xp11.2 tRCC, which were diagnosed by immunohistochemistry staining of TFE3 and fluorescence in situ hybridization at our center, were retrospectively reviewed. The 34 patients included 21 females and 13 males aged 3 to 64 years (median age: 27 years). Four patients were children or adolescents (<18 years of age), and 26 patients were young or middle-aged adults (18–45 years). Radical nephrectomy was performed on 25 patients. Laparoscopic nephron-sparing surgery was performed on 9 patients who presented with an isolated mass with a small diameter (<7 cm) and well-defined boundary on computed tomography imaging. Postoperative staging showed that 25 cases (73.53%) were at stage I/II, while 9 cases (26.47%) were at stage III/IV. All stage I/II patients received a favorable prognosis with a three-year overall survival rate of 100%, including the patients who underwent laparoscopic nephron-sparing surgery. With the exception of 2 children, the other 7 stage III/IV patients died or developed recurrence with a median follow-up of 29 months. On univariate analysis, maximum diameter, adjuvant treatment, TNM stage, lymph node metastasis, inferior vena cava tumor thrombosis and tumor boundary were identified as statistically significant factors impacting survival (P<0.05). Multivariate analysis indicated that TNM stage and inferior vena cava tumor thrombosis were independent prognostic factors (P<0.05). In conclusion, Xp11.2 tRCC is a rare subtype of renal cell carcinoma that mainly occurs in young females. Nephron-sparing surgery was confirmed effective preliminarily in the treatment of small Xp11.2 tRCCs with clear rims. Advanced TNM stage and inferior vena cava tumor thrombosis were associated with poor prognosis.

# Introduction

Since being recognized as a distinct entity by the World Health Organization (WHO) in 2004 [1], Xp11.2 translocation renal cell carcinoma (Xp11.2 tRCC) has attracted broad attention [2–5]. The term of Xp11.2 tRCC derived from several different chromosomal translocations of Xp11.2 breakpoints and formatting of TFE3 fusion gene, which resulted in a significant over-expression of TFE3 protein in tumor cells. In recent years, renal cell carcinoma associated with t(6;11)(p21;q12)/TFEB gene fusions has been found to share similar pathology, epidemiology and genetics characteristics with Xp11.2 tRCC[6]. At the same time, both TFE3 and TFEB are members of microphthalmia-associated transcription (MiT) factor family. On the basis of these findings, WHO in 2016 newly designated Xp11.2 tRCC as MiT family translocation renal cell carcinoma [7].

Microscopically, Xp11.2 tRCC is similar to clear cell renal cell carcinoma (CCRCC) or papillary renal cell carcinoma (PRCC) [4, 8], which makes it difficult for pathologists to distinguish Xp11.2 tRCC from other tumor types by histological characteristics. Although immunohistochemical staining for TFE3 (TFE3-IHC) serves as the basic method for the diagnosis of Xp11.2 tRCC, numerous reports have shown that TFE3-IHC has fairly high false-positive rates and low predictive values, which results in misdiagnoses in patients [4, 9–11]. To date, TFE3 break-apart fluorescence in situ hybridization (FISH) is regarded as the best method to diagnose Xp11.2 tRCC due to its advantages of high sensitivity and specificity [2, 12–15]. In the current study, 34 cases of Xp11.2 tRCC were diagnosed using a TFE3 break-apart FISH probe. To assess whether ASPL-TFE3 RCC, one of the subtypes of Xp11.2 tRCC with a fusion pattern of t(x;17)(p11.2;q25), showed more aggressive progress than other subtypes, we used an ASPL-TFE3 dual-fusion FISH probe for the diagnosis of ASPL-TFE3 RCC. Both the TFE3 break-apart FISH probe and ASPL-TFE3 dual-fusion FISH probe were demonstrated to identify the TFE3 and ASPL-TFE3 fusion genes, respectively, in our previous investigations [11, 15].

Despite its low incidence, Xp11.2 RCC is more harmful than conventional RCC because the majority of patients present at advanced stages and invasive clinical courses [2–5, 16]. Surgical treatment, especially radical nephrectomy (RN), remains the most common strategy to treat Xp11.2 tRCC. The implementation of nephron-sparing surgery (NSS) in Xp11.2 RCC is rarely reported, although it has been confirmed safe and effective in conventional RCC by several large retrospective studies [17, 18]. Herein, the outcomes of patients receiving RN or NSS were compared to ascertain the effectiveness of NSS for these potentially aggressive tumors.

# Patients and Methods

## Patients and diagnosed methods

The RCC patients were reviewed at Nanjing Drum Tower Hospital from January 2007 to February 2016, and the study was conducted from February to June 2016. All procedures were approved by the Medical Ethics Committee for human Experiments of Nanjing Drum Tower Hospital. This study involved the preoperative computed tomography (CT) characteristics, medical records, follow-ups and outcomes of patients. IHC staining was performed on formalin-fixed paraffin-embedded tissue sections with a TFE3 antibody, and 2 (+) to 3 (+) nuclear TFE3 immunoreactivity in more than 10% of cells was considered positive. Polyclonal break-apart probes for TFE3 gene rearrangement at the Xp11.2 region were utilized on samples from patients who had a positive TFE3-IHC result on formalin-fixed paraffin-embedded tissue microarray slides. Of the 1,239 RCC patients, 82 cases showed positive reaction to TFE3-IHC, and 34 cases were eventually diagnosed as Xp11.2 tRCC by FISH. With the exception of one

young female patient who lost to follow-up at the 88th postoperative month, the other 33 cases were followed over a period ranging from 3 to 104 months. The clinicopathological characteristics and follow-up information of these patients are shown in Table 1. To ascertain specific types of genetic changes in tumor cells, 23 cases (case 1 to case 23 in Table 1) were subjected to the ASPL-TFE3 dual-fusion FISH assay, but case 24 to case 34 were not achieved due to the absent of tissue sections. All the patients have signed in informed written consents to have their medical record data used in research.

## Evaluation of clinical data

The clinical data consisted of epidemiological features (gender and age), clinical manifestations, general history, preoperative CT imaging (laterality, tumor size and boundary), treatment methods (surgery, immune therapy or targeted therapy), pathological features (TNM stages, TFE3-IHC staining and FISH results) and clinical outcomes (normal, recurrent or dead). All of these data for the 34 patients were retrospectively analyzed. TNM staging was reclassified in accordance with the 7th American Joint Committee on Cancer (AJCC) staging criteria (2010). All of the patients were followed every 3 months during the first year, every 6 months during the following 4 years, and annually after 5 years until the time of death or loss to follow-up.

## Statistical analyses

Progression-free survival (PFS) was defined from the initiation of surgery to the date of disease progression or censoring at the time of last follow-up. Overall survival (OS) was defined as the time interval between the date of surgery and the date of death or last follow-up. PFS and OS curves were obtained by Kaplan–Meier analysis, and statistical comparisons were performed using the log-rank test. Multivariate Cox regression model was used to evaluate the predictive role of the factors that showed significance in the long-rank test. Univariate and multivariate analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). GraphPad Prism software version 5.0 was used to generate the survival curves. P-values $<0.05$ were considered statistically significant.

## Results

In total, 34 cases were identified encompassing 13 males and 21 females with a median age of 27 years (range of 3–64 years). Females with Xp11.2 tRCC in the age range of 18–45 years accounted for 81% of the patient population (17/21). With the exception of one patient who had a history of microcarcinoma thyroid in the previous 2 years, none of the patients had a history of tumors or chemotherapy. Nine of 34 (26%) patients underwent laparoscopic nephron-sparing surgery (LNSS) for the primary tumor, and the remaining 25 (74%) patients underwent open radical nephrectomy (ORN) or laparoscopic radical nephrectomy (LRN). Three of these latter patients who had inferior vena cava tumor thrombosis (cases 5, 6 and 10) underwent vena caval tumor thrombectomy simultaneously. The preoperative CT images for patients who underwent LNSS showed an isolated mass with a small diameter (<7 cm) and well-defined boundary.

Postoperative AJCC staging showed that 25 cases were classified as stage I/II, while 9 cases were classified as stage III/IV. Thirty-four patients were followed with a mean time of 41 months (range was 3–104 months). Twenty-four of the stage I/II cases received satisfactory results during a mean follow-up time of 42 months, except for one stage I male who was diagnosed with lung metastasis at the 11th postoperative month. However, for the stage III/IV patients, 7 adults who received surgery and postoperative adjuvant molecular-targeted therapy

**Table 1. Clinical data of 34 cases of Xp 11.2 tRCC.**

| Case | Age (years)/Sex/ Laterality | Symptom | Tumor size (cm) | Operation | Final diagnosis | ACJJ stage | Adjuvant therapy | Follow-up (months) and outcome |
|---|---|---|---|---|---|---|---|---|
| 1 | 21/F/R | Symptomless | 4 | LRN | ASPL-TFE3 RCC | pT1aN0M0, I | None | 60, Normal |
| 2 | 25/M/R | Gross hematuria, Flank pain | 7.1 | LRN | ASPL-TFE3 RCC | pT2aN0M0, II | IT | 18, Normal |
| 3 | 35/M/R | Symptomless | 6 | LRN | Xp11.2 RCC | pT1bN0M0, I | TMT | 50, Lung metastasis in 11 months, stable now |
| 4 | 26/M/L | Symptomless | 3.7 | ORN | Xp11.2 RCC | pT1aN0M0, I | TMT | 74, Normal |
| 5 | 39/F/R | Symptomless | 13 | ORN +VCTER | Xp11.2 RCC | pT3bN1M0, III | TMT | 25, Died of liver and brain metastasis |
| 6 | 46/F/R | Flank pain, Abdominal mass | 5.8 | ORN +VCTER | Xp11.2 RCC | pT3cN0M0, III | TMT | 15, Died of lung metastasis |
| 7 | 22/F/R | Gross hematuria | 3.9 | LRN | ASPL-TFE3 RCC | pT1aN1M0, III | TMT | 62, Died of bone metastasis |
| 8 | 26/F/R | Gross hematuria | 5 | LRN | Xp11.2 RCC | pT1bN0M0, I | None | 96, Normal |
| 9 | 7/M/L | Gross hematuria | 3 | ORN | ASPL-TFE3 RCC | pT1aN0M0, I | None | 104, Normal |
| 10 | 36/F/R | Gross hematuria | 8.6 | ORN +VCTER | ASPL-TFE3 RCC | pT3cN1M0, III | TMT | 33, Died of liver metastasis |
| 11 | 30/F/R | Symptomless | 3.2 | RA+LNSS | Xp11.2 RCC | pT1aNxM0, I | TMT | 63, Normal |
| 12 | 7/M/L | Gross hematuria, Abdominal mass | 10 | ORN | Xp11.2 RCC | pT4N1M0, IV | None | 65, Normal |
| 13 | 25/F/L | Symptomless | 3.8 | LRN | Xp11.2 RCC | pT1aN0M0, I | IT | 58, Normal |
| 14 | 24/F/R | Symptomless | 3.9 | LRN | Xp11.2 RCC | pT1aN0M0, I | IT | 42, Normal |
| 15 | 51/F/R | Symptomless | 5 | LNSS | Xp11.2 RCC | pT1bNxM0, I | IT | 53, Normal |
| 16 | 27/F/R | Gross hematuria, Flank pain | 6 | LRN | Xp11.2 RCC | pT1bN0M0, I | IT | 53, Normal |
| 17 | 26/M/L | Symptomless | 3.7 | LNSS | Xp11.2 RCC | pT1aN0M0, I | IT | 18, Normal |
| 18 | 3/F/R | Gross hematuria | 4 | ORN | Xp11.2 RCC | pT1aN1M0, III | None | 71, Normal |
| 19 | 11/F/R | Gross hematuria, Abdominal mass | 5.6 | ORN | Xp11.2 RCC | pT1bN0M0, I | None | 88, Lost |
| 20 | 40/M/L | Symptomless | 3.9 | LRN | Xp11.2 RCC | pT1aN0M0, I | None | 37, Normal |
| 21 | 19/F/L | Symptomless | 5 | LRN | Xp11.2 RCC | pT1bN0M0, I | None | 24, Normal |
| 22 | 38/M/R | Gross hematuria, Flank pain | 3 | LRN | ASPL-TFE3 RCC | pT1aN0M0, I | IT | 28, Normal |
| 23 | 29/M/L | Symptomless | 3.5 | LNSS | Xp11.2 RCC | pT1aN0M0, I | IT | 10, Normal |
| 24 | 25/F/R | Gross hematuria | 8.1 | LRN | Xp11.2 RCC | pT2aN0M0, II | None | 91, Normal |
| 25 | 27/M/L | Symptomless | 8.5 | LRN | Xp11.2 RCC | pT3aN0M0, III | TMT | 24, Recurred in 16 months |
| 26 | 22/F/R | Gross hematuria | 5 | LRN | Xp11.2 RCC | pT1bN0M0, I | IT | 38, Normal |
| 27 | 45/M/R | Symptomless | 5.5 | LNSS | Xp11.2 RCC | pT1bNxM0, I | IT | 14, Normal |
| 28 | 25/F/R | Gross hematuria | 3.5 | LRN | Xp11.2 RCC | pT1aNxM0, I | IT | 13, Normal |
| 29 | 39/F/R | Flank pain | 4.5 | LNSS | Xp11.2 RCC | pT1bNxM0, I | IT | 11, Normal |
| 30 | 45/F/L | Symptomless | 12 | ORN | Xp11.2 RCC | pT3aN0M0, III | TMT | 30, Recurred in 12 months |
| 31 | 30/F/L | Symptomless | 9.5 | LRN | Xp11.2 RCC | pT3aN0M0, III | TMT | 20, Recurred in 14 months |
| 32 | 64/M/L | Symptomless | 3 | LNSS | Xp11.2 RCC | pT1aN0M0, I | IT | 3, Normal |
| 33 | 55/F/R | Symptomless | 3 | LNSS | Xp11.2 RCC | pT1aN0M0, I | IT | 8, Normal |

(*Continued*)

**Table 1.** (*Continued*)

| Case | Age (years)/Sex/ Laterality | Symptom | Tumor size (cm) | Operation | Final diagnosis | ACJJ stage | Adjuvant therapy | Follow-up (months) and outcome |
|---|---|---|---|---|---|---|---|---|
| 34 | 42/M/L | Symptomless | 3.5 | LNSS | Xp11.2 RCC | pT1aN0M0, I | IT | 4, Normal |

ACJJ: American Joint Committee on Cancer; F: Female; IT: Immune therapy; L: Left; LNSS: laparoscopic nephron-sparing surgery; LRN: Laparoscopic radical nephrectomy; M: Male; ORN: Open radical nephrectomy; R: Right; RA: Radiofrequency Ablation; TMT: Targeted molecular therapy; VCTER: Vena cava tumor embolus resection.

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(receptor tyrosine kinase inhibitor sunitinib or sorafinib) developed distant metastases or local recurrence, while two children who underwent surgery alone did not show disease progression during 65 and 71 months of follow-up. Of the three recurrent patients, case 25 was found abdominal wall recurrence, and case 30 along with case 31 were found ipsilateral abdominal cavity recurrence. Secondary surgeries were preformed to resect the recurrent lesions. Unfortunately, there was still one patient (case 25) who experienced an additional recurrence. Importantly, 9 patients who underwent LNSS showed no evidence of progress with a mean follow-up of 20 months.

Of the 34 cases diagnosed as Xp11.2 tRCC by the use of FISH, 7 cases (21%) were originally misdiagnosed as CCRCC (2 cases), PRCC (4 cases), and unclassified renal cell carcinoma (1 case).

Table 2 summarizes each tested variable and the significance of variation. Univariate analysis showed that adjuvant treatment, TNM stage, regional lymph node metastasis and vena cava tumor thrombosis were risk factors for OS. In addition, maximum diameter, adjuvant treatment, TNM stage and vena cava tumor thrombosis were identified as risk factors for PFS (all $P < 0.05$). The Kaplan-Meier survival curves for OS and PFS according to TNM stage are shown in Fig 1. The OS and PFS curves according to vena cava tumor thrombosis are shown in Fig 2. The four pairs of PFS and OS curves depending on TNM stage and vena cava tumor thrombosis were distinctly tiered and statistically significant.

Multivariate analysis was performed using a Cox proportional hazards model to assess the independent prognostic value of clinicopathological features for PFS and OS. Considering that adjuvant treatment was closely associated with postoperative TNM stage, we excluded it when performing multivariate analysis to obtain a more reliable result. The Cox regression analysis result for PFS is shown in Table 3. TNM stage ($P = 0.015$) and vena cava tumor thrombosis ($P = 0.024$) showed independent prognostic significance. However, Cox regression indicated no significant independent prognostic factor when analyzing OS.

## Discussion

Xp11.2 tRCC typically affects children and young adults under 45 years of age [3, 4, 19, 20] with a one-third incidence in juveniles and 0.2–5.0% incidence in adults [16, 21–23]. Unlike pediatric RCCs, cytogenetics is not routinely performed for adult RCCs due to the relatively lower incidence rate, which results in most misdiagnoses as conventional RCCs. Therefore, the exact frequency in adults is underestimated [21, 22], as inferred from our investigation. Compared with standard cytogenetics, TFE3-IHC has advantages of rapid diagnosis, high economy and high sensitivity in diagnosing Xp11.2 RCC. Thus, TFE3-IHC can be performed proactively as a screening test, and FISH can be performed as a verification test. Hirobe and Masumori suggested that the combination of TFE3-IHC and FISH is an effective method to diagnose Xp11.2 tRCCs, which can improve specificity and potentially eliminate false-positives resulting from overstaining [4].

**Table 2. Kaplan-Meier univariate analysis of prognostic factors for overall survival (OS) and progression-free survival (PFS).**

| Variable | | Number (%) | One-year overall survival rate | Three-year overall survival rate | Five-year overall survival rate | P value (Long-rank test) OS | PFS |
|---|---|---|---|---|---|---|---|
| Age | | | | | | 0.230 | 0.192 |
| | <18 years | 4(11.8%) | 100% | 100% | 100% | | |
| | ≥18 years | 30(88.2%) | 96% | 84% | 60% | | |
| Sex | | | | | | 0.182 | 0.554 |
| | Male | 13(38.2%) | 100% | 100% | 100% | | |
| | Female | 21(61.8%) | 94% | 86% | 81% | | |
| Symptom | | | | | | 0.753 | 0.211 |
| | Gross hematuria | 13(38.2%) | 100% | 90% | 68% | | |
| | Non-gross hematuria | 21(61.8%) | 93% | 84% | 84% | | |
| Laterality | | | | | | 0.172 | 0.967 |
| | Left | 13(38.2%) | 94% | 81% | 61% | | |
| | Right | 21(61.8%) | 100% | 100% | 100% | | |
| Operation | | | | | | 0.488 | 0.179 |
| | Radical nephrectomy | 25(73.5%) | 96% | 86% | 69% | | |
| | Partial nephrectomy | 9(26.5%) | 100% | 100% | 100% | | |
| Maximum diameter | | | | | | 0.199 | **0.007** |
| | ≤7 cm | 26(76.5%) | 95% | 95% | 74% | | |
| | >7 cm | 8(23.5%) | 100% | 60% | 60% | | |
| Adjuvant treatment | | | | | | **0.016** | **<0.001** |
| | Without | 9(26.5%) | 100% | 100% | / | | |
| | Immune therapy | 15(44.1%) | 100% | 100% | / | | |
| | Targeted molecular therapy | 10(29.4%) | 89% | 64% | 32% | | |
| TNM stage | | | | | | **0.002** | **<0.001** |
| | Staged I / II | 25(73.5%) | 100% | 100% | 100% | | |
| | Staged III / IV | 9(26.5%) | 88% | 59% | 29% | | |
| Lymph node metastasis | | | | | | **0.018** | 0.080 |
| | Positive | 5(14.7%) | 100% | 60% | 30% | | |
| | Negative | 29(85.3%) | 95% | 95% | 95% | | |
| Inferior vena cava tumorthrombosis | | | | | | **<0.001** | **<0.001** |
| | Positive | 3(8.8%) | 67% | / | / | | |
| | Negative | 31(91.2%) | 100% | 100% | 82% | | |
| Fusion type | | | | | | 0.280 | 0.439 |
| | ASPL | 6(26.1%) | 100% | 78% | 39% | | |
| | Non-ASPL | 17(73.9%) | 94% | 87% | 87% | | |
| Boundary | | | | | | 0.088 | **0.048** |
| | Clear | 15(44.1%) | 100% | 100% | 100% | | |
| | Not clear | 19(55.9%) | 94% | 80% | 48% | | |

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In the current study, a predominance of young females and right side prevalence was observed, which is consistent with previous reports [4, 22, 24]. In the report by Argani on 28 cases of Xp11.2 tRCC, there was a strong female (22 cases, 79%) and a slight right (14/22, 64%) preference, of which 22 cases (79%) were younger than 45 years [22]. Choueiri et al. identified 15 Xp11.2 tRCCs, and female patients accounted for 80% [24]. Qu diagnosed 30 cases of Xp11.2 tRCC and reported that 18 cases (60%) were females, with 1/3 aged between 18 and 45
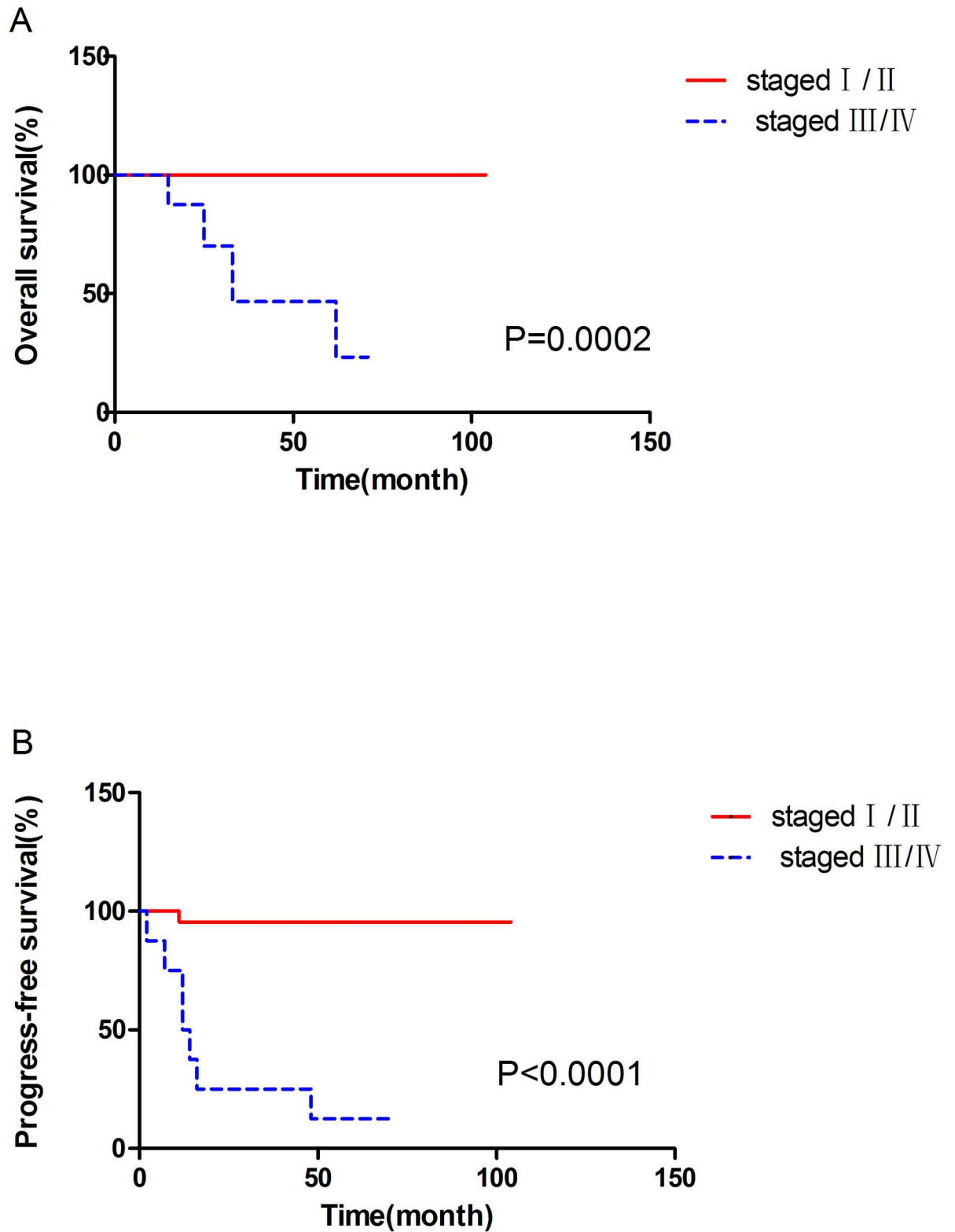
Fig 1. Survival curves of overall survival (A) and progression-free survival (B) between stage I/II and stage III/IV patients. The overall survival and progression-free survival between stage I/II and stage III/IV in Xp11.2 tRCC were statistically significant.
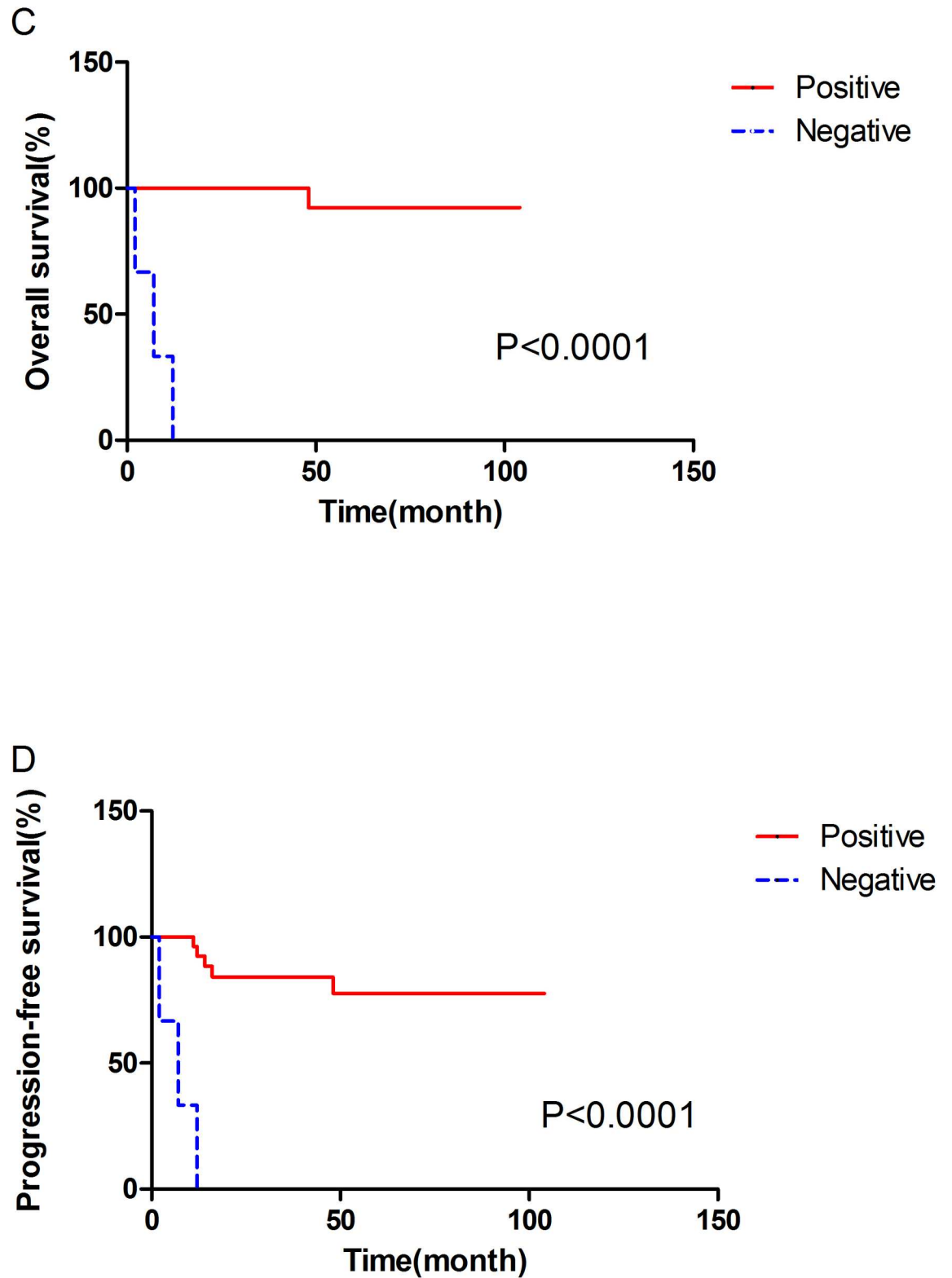
C



D



**Fig 2. Survival curves of overall survival (C) and progression-free survival (D) between inferior vena cava tumor thrombosis-positive and -negative patients.** The overall survival and progression-free survival between inferior vena cava tumor thrombosis-positive and -negative patients were statistically significant.

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Table 3. Multivariate Cox regression analyses of progression-free survival.

| Variable | B | SE | Wald value | P value | HR (95%CI) |
|---|---|---|---|---|---|
| Maximum diameter | -0.056 | 0.159 | 0.124 | 0.725 | 0.693–1.291 |
| TNM stage | 3.102 | 1.270 | 5.962 | **0.015** | 1.844–268.066 |
| Lymph node metastasis | -1.492 | 1.003 | 2.212 | 0.137 | 0.031–1.607 |
| Inferior vena cava tumor thrombosis | 2.792 | 1.233 | 5.129 | **0.024** | 1.456–182.861 |
| Boundary | 11.613 | 234.618 | 0.002 | 0.961 | 0.000–5.635 |

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years and 17 of 30 cases showing a right side prevalence [2]. One probable explanation for the female predominance might be that females possess two X chromosomes while males only possess one, which leads to a higher incidence of X chromosome translocation. It remains unknown why the right side showed predominance. Due to an insufficient sample size, the actual frequency and mechanisms require further investigation.

With its aggressive biological behavior and local invasion tendency in adult, Xp11.2 tRCC trends to present with lymphatic and distant organ metastasis at diagnosis. To date, there is not yet a consensus regarding therapy, RN is recommended if possible [8]. Nevertheless, with the popularly utilized of NSS in recent year, part of the early stage Xp11.2 tRCC patients received NSS. Urologists and patients usually puzzled with the management between a completion nephrectomy and close surveillance when a patient was incidentally diagnosed with Xp11.2 tRCC after a partial nephrectomy. However, results from published studies reveal that the practice of NSS also produces favorable treatment outcomes in short-term follow-up studies [3, 18, 25]. Gorin and Ball reported that 4 patients who were treated with NSS remained alive without evidence of disease during a mean follow-up of 37 months [18]. Lim and You retrospectively analyzed 8 cases of Xp11.2 tRCC, and all of these patients underwent open or robotic NSS and were disease-free with a follow-up time of 18 to 47.6 months [3]. Herein, the treatment of Xp11.2 RCC by LNSS obtained a satisfactory result with a mean follow-up of 20 months. Even though, long-time follow-up is need as its well documented ability of recurrent late, especially PRCC-TFE3 RCC [5, 26].

Because pathological types are usually uncertain before surgery, radiologic and ultrasonic information may be the only reference to determine whether RN or NSS should be performed if a patient has a renal tumor. According to the literature, isolated masses with small diameters (<7 cm) and well-defined boundaries are regarded as appropriate candidates for NSS [17, 20, 27, 28], which is based on the finding that tumors with small diameters and clear rims predicate positive prognosis. From our statistical outcomes, both small diameters (<7 cm) and clear boundaries were protective factors for prognosis. Furthermore, Xiangming and colleagues reported that there was an inverse correlation between diameters and boundary, with larger diameters showing more indefinite boundaries [25]. As NSS offers oncological outcomes and preserves renal function comparable with that of RN, NSS should be considered if a young patient presents with a small lesion (<7 cm) and distinct boundary, especially patients with anatomical or a functional solitary kidney.

Since chemotherapy and radiotherapy proved to be invalid for Xp11 tRCC, the optimal and reliable adjuvant treatment remains to be developed. According to several previous studies, interferon (IFN-a) and interleukin-2 (IL-2) do not produce a significant response in this type of tumor [21, 29]. Recently, the molecular-targeted therapy using a receptor tyrosine kinase inhibitor, e.g., sunitinib or sorafinib, was shown to be a treatment choice for patients with lymph node or distant metastases [24, 30]. Choueiri et al. retrospectively reviewed 15 adult patients with metastatic Xp11.2 tRCC, and they reported that 3 patients achieved a partial response, 7 patients had stable disease, and 5 patients developed progressive disease [24].

However, the patients in Choueiri's study were diagnosed by TFE3-IHC. In the study of Malouf, the researchers examined 21 cases of metastatic Xp11.2 tRCCs who received targeted therapy, and they reported that 7 patients achieved an objective response to sunitinib with a median PFS of 8.2 months compared to cytokine therapy with a median PFS of 2 months. In the current study, a 35-year-old male presented with lung metastasis at 11 months after surgery, and this patient then received targeted therapy for 8 weeks and survived during the postoperative follow-up of 50 months. However, all stage III adults who received ORN/LRN and targeted molecular therapy ultimately developed terminal or recurrent cancer. Giving the toxicity to growth, the usage of targeted therapy on juvenilities is usually limited, although trials have been reported [30, 31].

The literature shows that adults with Xp11 tRCC possess a grimmer prognosis than children, which is congruent with the data from our study, despite our finding from the univariate analysis indicated that age was not associated with prognosis due to the small sample size. For pediatrics, regional lymph node metastases do not necessarily predict poor prognosis [20, 23, 28, 32]. Geller et al. retrospectively reviewed 58 patients and found that children with lymph node-positive RCC in the absence of distant metastatic disease had a relatively favorable long-term prognosis with a survival rate (72.4%) nearly triple that of the adult controls [33]. Song et al. studied 22 cases of pediatric Xp11.2 tRCCs, including 12 stage III/IV patients, and reported that 13 patients were still alive and 2 had a terminal course with a postoperative follow-up of 6 months to 35 years [20]. Nevertheless, a meta-analysis showed that Xp11.2 tRCCs showed a poorer prognostic than non-Xp11.2 translocation carcinomas in children and young adults [34].

Similar to conventional RCCs, advanced TNM stage and inferior vena cava tumor thrombosis are the most significant factors that predict poor prognosis in Xp11 tRCC, which was verified by univariate and multivariate analyses in our study. Recently, Qu et al. [4] reported 30 cases of Xp11.2 tRCC diagnosed by FISH and showed that 11 of 14 stage III/IV patients presented with metastases of lung, liver or other organs, which was in contrast to the remaining 16 stage I/II patients. In the reports of Nesbitt et al., 20 of 37 patients with RCC with inferior vena cava tumor thrombus were alive after surgical resection, and the overall 2- and 5-year survival rates were 61.7% and 33.6%, respectively [35]. Nevertheless, the three patients with inferior vena cava tumor thrombosis in our investigation showed distant metastases and rapid termination of their life with a mean follow-up of 33 months, regardless of having undergone vena caval tumor thrombectomy and targeted molecular therapy.

In our investigation, 2 out of 6 ASPL-TFE3 RCC patients died due to multiple metastases. In the study by Ellis et al. [5], all of the published ASPL-TFE3 and PRCC-TFE3 cases were reviewed, and they reported that 24 out of 32 ASPL-TFE3 cases showed regional lymph node involvement, which was a higher incidence rate than that of PRCC-TFE3 cases. In the study by Komai et al. [36], 2 cases with ASPL-TFE3 RCC displayed metastasis, and one patient died of the disease. However, comparison of the survival time of Xp11.2 tRCC between ASPL fusion and non-ASPL fusion cases showed no difference by long-rank test in our study.

As its low-frequency, the reported Xp11.2 tRCCs which were diagnosed by FISH or PCR less than 150 all over the world. To date, this is one of the largest single-center clinical report on Xp11.2 tRCC confirmed by FISH. The data we published will undoubtedly enrich the literature base. There are some limitations to the current study. First, the sample size was insufficient due to the low incidence of this rare disease. Although the TFE3-IHC assay is sensitive to detect TFE3 in the screening of Xp11.2 tRCC, potential missed diagnoses may still exist. Second, the follow-up time was relatively short, especially for patients treated with NSS. Long-term follow-up is needed to assess the therapeutic effects of NSS. Third, only 6 cases were identified as genetic fusion types by the ASPL-TFE3 dual-fusion FISH assay, which did not allow

the comparison between different subtypes of Xp11.2 tRCC to be performed. We are currently utilizing additional types of fusion probes to identify the genetic fusion types of those cases.

## Conclusions

Xp11.2 tRCC is a rare subset of RCC that mainly occurs in children and young females with gross hematuria. The combination of the TFE3-IHC assay and FISH analysis is an accurate and effective approach to screen and confirm, respectively, the diagnosis of Xp11.2 tRCC. Radical and partial nephrectomy constitute alternative treatments for patients with well-defined lesions measuring <7 cm. The prognosis of adults is significantly inferior to that of children, especially for patients at advanced TNM stages and with inferior vena cava tumor thrombosis.

## Supporting Information

**S1 Table. The STROBE checklist of the current study.** This checklist contains items that reflected in our study according to STROBE guidelines.
(DOCX)

## Author Contributions

**Conceptualization:** WG HG DL.

**Data curation:** NL ZW XC.

**Formal analysis:** NL LX XC.

**Funding acquisition:** WG HG DL.

**Investigation:** NL XC LX.

**Methodology:** WG XC.

**Project administration:** BM ZW.

**Resources:** WG HG DL.

**Software:** NL LX.

**Supervision:** NL WG.

**Validation:** BM XC.

**Visualization:** NL ZW BM WG.

**Writing – original draft:** NL ZW.

**Writing – review & editing:** WG LX BM.

## References

1. Eble JN, Sauter G, Epstein JI, Sesterhenn IA. Pathology and genetics. Tumors of the urinary system and male genital organs. Lyon: IARC Press; 2004.

2. Qu Y, Gu C, Wang H, Chang K, Yang X, Zhou X, et al. Diagnosis of adults Xp11.2 translocation renal cell carcinoma by immunohistochemistry and FISH assays: clinicopathological data from ethnic Chinese population. Sci Rep. 2016; 6:21677. doi: 10.1038/srep21677 PMID: 26880493

3. Lim B, You D, Jeong IG, Kwon T, Hong S, Song C, et al. Clinicopathological features of Xp11.2 translocation renal cell carcinoma. Korean J Urol. 2015 Mar; 56(3):212–7. doi: 10.4111/kju.2015.56.3.212 PMID: 25763125

4. Hirobe M, Masumori N, Tanaka T, Kitamura H, Tonooka A, Hasegawa T, et al. Clinicopathological characteristics of Xp11.2 translocation renal cell carcinoma in adolescents and adults: diagnosis using immunostaining of transcription factor E3 and fluorescence in situ hybridization analysis. Int J Urol. 2015 Nov 13; 23(2):140–5. doi: 10.1111/iju.13007 PMID: 26563505

5. Ellis CL, Eble JN, Subhawong AP, Martignoni G, Zhong M, Ladanyi M, et al. Clinical heterogeneity of Xp11 translocation renal cell carcinoma: impact of fusion subtype, age, and stage. Mod Pathol. 2014 Jun; 27(6):875–86. doi: 10.1038/modpathol.2013.208 PMID: 24309327

6. Argani P, Lae M, Hutchinson B, Reuter VE, Collins MH, Perentesis J, et al. Renal carcinomas with the t (6;11)(p21;q12): clinicopathologic features and demonstration of the specific alpha-TFEB gene fusion by immunohistochemistry, RT-PCR, and DNA PCR. Am J Surg Pathol. 2005 Feb; 29(2):230–40. PMID: 15644781

7. Moch H, Humphrey PA, Ulbright TM, Reuter V. WHO Classification of Tumours of the Urinary System and Male Genital Organs. Lyon, France: International Agency for Research on Cancer; 2016.

8. Kuroda N, Mikami S, Pan CC, Cohen RJ, Hes O, Michal M, et al. Review of renal carcinoma associated with Xp11.2 translocations/TFE3 gene fusions with focus on pathobiological aspect. Histol Histopathol. 2012 Feb; 27(2):133–40. PMID: 22207547

9. Klatte T, Streubel B, Wrba F, Remzi M, Krammer B, de Martino M, et al. Renal cell carcinoma associated with transcription factor E3 expression and Xp11.2 translocation: incidence, characteristics, and prognosis. Am J Clin Pathol. 2012 May; 137(5):761–8. doi: 10.1309/AJCPQ6LLFMC4OXGC PMID: 22523215

10. Gaillot-Durand L, Chevallier M, Colombel M, Couturier J, Pierron G, Scoazec JY, et al. Diagnosis of Xp11 translocation renal cell carcinomas in adult patients under 50 years: interest and pitfalls of automated immunohistochemical detection of TFE3 protein. Pathol Res Pract. 2013 Feb 15; 209(2):83–9. doi: 10.1016/j.prp.2012.10.013 PMID: 23246378

11. Chen X, Yang Y, Gan W, Xu L, Ye Q, Guo H. Newly designed break-apart and ASPL-TFE3 dual-fusion FISH assay are useful in diagnosing Xp11.2 translocation renal cell carcinoma and ASPL-TFE3 renal cell carcinoma: a STARD-compliant article. Medicine. 2015 May; 94(19):e873. doi: 10.1097/MD.0000000000000873 PMID: 25984679

12. Rao Q, Williamson SR, Zhang S, Eble JN, Grignon DJ, Wang M, et al. TFE3 break-apart FISH has a higher sensitivity for Xp11.2 translocation-associated renal cell carcinoma compared with TFE3 or cathepsin K immunohistochemical staining alone: expanding the morphologic spectrum. Am J Surg Pathol. 2013 Jun; 37(6):804–15. doi: 10.1097/PAS.0b013e31827e17cb PMID: 23598965

13. Green WM, Yonescu R, Morsberger L, Morris K, Netto GJ, Epstein JI, et al. Utilization of a TFE3 break-apart FISH assay in a renal tumor consultation service. Am J Surg Pathol. 2013 Aug; 37(8):1150–63. doi: 10.1097/PAS.0b013e31828a69ae PMID: 23715164

14. Hodge JC, Pearce KE, Wang X, Wiktor AE, Oliveira AM, Greipp PT. Molecular cytogenetic analysis for TFE3 rearrangement in Xp11.2 renal cell carcinoma and alveolar soft part sarcoma: validation and clinical experience with 75 cases. Mod Pathol. 2014 Jan; 27(1):113–27. doi: 10.1038/modpathol.2013.83 PMID: 23828314

15. Chen X, Gan W, Ye Q, Yang J, Guo H, Li D. [Application of polyclonal break-apart probes in the diagnosis of Xp11.2 translocation renal cell carcinoma]. Zhonghua Yi Xue Za Zhi. 2014 Dec 16; 94(46):3675–7. PMID: 25622964

16. Sukov WR, Hodge JC, Lohse CM, Leibovich BC, Thompson RH, Pearce KE, et al. TFE3 rearrangements in adult renal cell carcinoma: clinical and pathologic features with outcome in a large series of consecutively treated patients. Am J Surg Pathol. 2012 May; 36(5):663–70. doi: 10.1097/PAS.0b013e31824dd972 PMID: 22498819

17. Carini M, Minervini A, Lapini A, Masieri L, Serni S. Simple enucleation for the treatment of renal cell carcinoma between 4 and 7 cm in greatest dimension: progression and long-term survival. J Urol. 2006 Jun; 175(6):2022–6; discussion 6. doi: 10.1016/S0022-5347(06)00275-8 PMID: 16697790

18.  Gorin MA, Ball MW, Pierorazio PM, Argani P, Allaf ME. Partial nephrectomy for the treatment of translocation renal cell carcinoma. Clin Genitourin Cancer. 2015 Jun; 13(3):e199–201. doi: 10.1016/j.clgc.2014.12.008 PMID: 25592300

19.  He J, Chen X, Gan W, Zhu B, Fan X, Guo H, et al. Renal cell carcinoma associated with Xp11.2 translocation/TFE3 gene fusions: clinical experience and literature review. Future Oncol. 2015 Dec; 11 (24):3243–52. doi: 10.2217/fon.15.177 PMID: 26169997

20.  Song HC, Sun N, Zhang WP, He L, Fu L, Huang C. Biological characteristics of pediatric renal cell carcinoma associated with Xp11.2 translocations/TFE3 gene fusions. J Pediatr Surg. 2014 Apr; 49(4):539–42. doi: 10.1016/j.jpedsurg.2013.10.005 PMID: 24726108

21.  Komai Y, Fujiwara M, Fujii Y, Mukai H, Yonese J, Kawakami S, et al. Adult Xp11 translocation renal cell carcinoma diagnosed by cytogenetics and immunohistochemistry. Clin Cancer Res. 2009 Feb 15; 15 (4):1170–6. doi: 10.1158/1078-0432.CCR-08-1183 PMID: 19228722

22.  Argani P, Olgac S, Tickoo SK, Goldfischer M, Moch H, Chan DY, et al. Xp11 translocation renal cell carcinoma in adults: expanded clinical, pathologic, and genetic spectrum. Am J Surg Pathol. 2007 Aug; 31 (8):1149–60. doi: 10.1097/PAS.0b013e318031ffff PMID: 17667536

23.  Geller JI, Argani P, Adeniran A, Hampton E, De Marzo A, Hicks J, et al. Translocation renal cell carcinoma: lack of negative impact due to lymph node spread. Cancer. 2008 Apr 1; 112(7):1607–16. doi: 10.1002/cncr.23331 PMID: 18278810

24.  Choueiri TK, Lim ZD, Hirsch MS, Tamboli P, Jonasch E, McDermott DF, et al. Vascular endothelial growth factor-targeted therapy for the treatment of adult metastatic Xp11.2 translocation renal cell carcinoma. Cancer. 2010 Nov 15; 116(22):5219–25. doi: 10.1002/cncr.25512 PMID: 20665500

25.  Cheng X, He J, Gan W, Fan X, Yang J, Zhu B, et al. Pseudocapsule of renal cell carcinoma associated with Xp11.2 translocation/TFE3 gene fusion: a clue for tumor enucleation? Int J Clin Exp Pathol. 2015; 8(5):5403–10. PMID: 26191243

26.  Argani P, Antonescu CR, Couturier J, Fournet JC, Sciot R, Debiec-Rychter M, et al. PRCC-TFE3 renal carcinomas: morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1)(p11.2;q21). Am J Surg Pathol. 2002 Dec; 26(12):1553–66. PMID: 12459622

27.  Minervini A, Tuccio A, Lapini A, Lanzi F, Vittori G, Siena G, et al. Review of the current status of tumor enucleation for renal cell carcinoma. Arch Ital Urol Androl. 2009 Jun; 81(2):65–71. PMID: 19760858

28.  Ramphal R, Pappo A, Zielenska M, Grant R, Ngan BY. Pediatric renal cell carcinoma: clinical, pathologic, and molecular abnormalities associated with the members of the mit transcription factor family. Am J Clin Pathol. 2006 Sep; 126(3):349–64. doi: 10.1309/98YE9E442AR7LX2X PMID: 16880148

29.  Nelius T, Al-Khalil I, Vordermark J, Rinard-Holden K, Cammack T, Mamlok V, et al. TFE3 Translocation-Associated Renal Cell Carcinoma Presenting as Avascular Necrosis of the Femur in a 19-Year-Old Patient: Case Report and Review of the Literature. Case Rep Med. 2011; 2011:432917. doi: 10.1155/2011/432917 PMID: 22007232

30.  Malouf GG, Camparo P, Oudard S, Schleiermacher G, Theodore C, Rustine A, et al. Targeted agents in metastatic Xp11 translocation/TFE3 gene fusion renal cell carcinoma (RCC): a report from the Juvenile RCC Network. Ann Oncol. 2010 Sep; 21(9):1834–8. doi: 10.1093/annonc/mdq029 PMID: 20154303

31.  Chowdhury T, Prichard-Jones K, Sebire NJ, Bier N, Cherian A, Sullivan MO, et al. Persistent complete response after single-agent sunitinib treatment in a case of TFE translocation positive relapsed metastatic pediatric renal cell carcinoma. J Pediatr Hematol Oncol. 2013 Jan; 35(1):e1–3. doi: 10.1097/MPH.0b013e318266bf34 PMID: 22995920

32.  Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, et al. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. Am J Pathol. 2001 Jul; 159(1):179–92. doi: 10.1016/S0002-9440(10)61684-7 PMID: 11438465

33.  Geller JI, Dome JS. Local lymph node involvement does not predict poor outcome in pediatric renal cell carcinoma. Cancer. 2004 Oct 1; 101(7):1575–83. doi: 10.1002/cncr.20548 PMID: 15378495

34.  Qiu R, Bing G, Zhou XJ. Xp11.2 Translocation renal cell carcinomas have a poorer prognosis than non-Xp11.2 translocation carcinomas in children and young adults: a meta-analysis. Int J Surg Pathol. 2010 Dec; 18(6):458–64. doi: 10.1177/1066896910375565 PMID: 20643670

35.  Nesbitt JC, Soltero ER, Dinney CP, Walsh GL, Schrump DS, Swanson DA, et al. Surgical management of renal cell carcinoma with inferior vena cava tumor thrombus. Ann Thorac Surg. 1997 Jun; 63 (6):1592–600. PMID: 9205155

36.  Malouf GG, Camparo P, Molinie V, Dedet G, Oudard S, Schleiermacher G, et al. Transcription factor E3 and transcription factor EB renal cell carcinomas: clinical features, biological behavior and prognostic factors. J Urol. 2011 Jan; 185(1):24–9. doi: 10.1016/j.juro.2010.08.092 PMID: 21074195