Clinicopathological Characterization of Renal Cell Carcinoma in Young Adults: a contemporary update and review of literature

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Abstract

Background

Renal cell carcinomas are relatively rare in children and young adults. While well characterized in adults, the morphologic and molecular characterization of these tumors in young patients is relatively lacking.

Objective

To explore the spectrum of renal cell carcinoma (RCC) subtypes in children and young adults and to determine their clinical-pathological, immunohistochemical and molecular characteristics, by evaluating a large retrospective cohort of renal cell carcinoma patients 30 years of age or younger.

Results

Sixty-eight cases with confirmed diagnosis of renal cell carcinoma at 30 years of age or younger were identified at our institution. Clear cell carcinoma accounted for the most common subtype seen in this age group. Translocation renal cell carcinoma and rare familial syndrome subtypes such as succinate dehydrogenase deficient renal cell carcinoma and Tuberous Sclerosis Complex- associated renal cell carcinoma were found relatively more frequently in this cohort. Despite applying the 2016 WHO classification criteria, a high proportion of the tumors in our series remained unclassified.

Conclusions

Our results suggest that renal cell carcinoma in children and young adults is a relatively rare disease that shares many histologic similarities to renal cell carcinoma occurring in adults and yet demonstrate some unique clinical-pathological differences. MiT family translocation RCC and rare familial syndrome subtypes are relatively more frequent in the pediatric and adolescent

age groups than in adults. Clear cell RCC still accounted for the most common subtype seen in this age group. MiT family translocation RCC patients presented with advanced stage disease and had poor clinical outcomes. The large and heterogeneous subgroup of unclassified renal cell carcinoma contains phenotypically distinct tumors with further potential for future subcategories in the renal cell carcinoma classification.

Introduction

Renal tumors comprise a diverse spectrum of neoplastic lesions with patterns that are relatively distinct for children and young adults. Adult renal cell carcinomas (RCCs) comprise 6% of all cancers with a peak incidence in the 6th decade. Clear cell renal cell carcinoma (CCRCC) is the predominant subtype in older patients [1]. In children and young adults, renal cell carcinomas are rare, accounting for 2% of pediatric renal tumors [2]. Pediatric renal cell carcinoma lacks the male: female gender predilection (2:1) as well as associations with environmental factors that are often present in adult renal tumors [3-5]. Additionally, prognosis and clinical outcomes have been reported to be significantly different between both age groups [6].

In recent years, significant advances have been made in our knowledge of the genomic background of renal cell carcinomas. As a result, the 2004 World Health Organization (WHO) [7] classification of renal tumors recognized new distinct entities including Xp11.2 translocation renal cell carcinoma and syndrome associated tumors. The morphologic spectrum was further expanded in the 2016 WHO classification [8] to include entities such as Hereditary Leiomyomatosis and renal cell carcinoma associated renal cell carcinoma (HLRCC- associated RCC), Succinate dehydrogenase- deficient renal cell carcinoma (SDH- deficient RCC), Tubulocystic renal cell carcinoma, Acquired cystic disease-associated renal cell carcinoma

(ACD-RCC) and Clear cell papillary renal cell carcinoma. While several studies have been previously published regarding the clinical features of renal cell carcinomas in young adults [9-11], the morphologic and molecular characterization of these tumors, especially in recognition of the recent 2016 WHO classification, are lacking.

In this study, we sought to explore the spectrum of renal cell carcinoma subtypes in children and young adults and to determine their clinicopathological, immunohistochemical and molecular characteristics, by evaluating a large retrospective cohort of renal cell carcinoma patients 30 years of age or younger.

Materials and methods

Case selection

After approval by the Institutional Review Board (IRB), a retrospective pathological and clinical review of renal cell carcinoma in patients who are 30 years of age or younger, diagnosed between January 1986 and December 2018 at the University of Michigan Health system was conducted. Only patients who underwent definitive surgical treatment by partial or radical nephrectomy with histologic material available for review were included. Electronic medical records and pathology reports were reviewed to analyze clinical parameters (age at diagnosis, sex, clinical stage, previous chemotherapy exposure, relevant family history, history of Von Hippel-Lindau (VHL) syndrome or other hereditary renal cell carcinoma syndromes, history of other tumors and end-stage renal disease), pathological variables (tumor size, focality and laterality) and follow up data (vital status and presence of metastasis/recurrence).

Representative hematoxylin and eosin-stained whole tissue sections of each tumor were reviewed by multiple study pathologists (EA, AU and RM) to evaluate the renal tumors per the current (2016) WHO renal tumor classification criteria [8]. Tumors recognized as CCRCC, papillary renal cell carcinoma (PRCC), Chromophobe RCC (ChRCC) and Renal medullary carcinoma had the usual morphologic features as previously described [7]. MiT family translocation renal cell carcinoma was considered by morphology if it displayed papillary or

pseudo-papillary architecture with distinctly voluminous cytoplasm in the majority of tumor cells; other morphologic appearances as described previously were taken into consideration as well [12-14]. SDH-deficient RCC was considered if tumors displayed the presence of cytoplasmic vacuoles and inclusion-like spaces containing eosinophilic fluid or flocculent material. Tuberous Sclerosis Complex- associated RCC (TSC-associated RCC), in patients with tuberous sclerosis, was classified if tumors displayed distinct morphologic features including features similar to tumors previously described as "renal angiomyoadenomatous tumor", features similar to ChRCC or showed a granular-macrocystic morphology [15]. Unclassified RCC was diagnosed when two different well-recognized histological features coexisted in different areas of the same gross tumor, or in cases where histological features and immunohistochemical profile were atypical or unusual, precluding classification into RCC subtypes described above.

Tumor grade was assigned according to the WHO/ISUP grading system [16] and was applied to CCRCC and PRCC. All cases were staged according to the American Joint Committee TNM Cancer Staging Manual, 8th Edition.

Immunohistochemistry

The renal tumors were placed into their appropriate diagnostic categories using appropriate immunohistochemical stains as/when necessary. Representative paraffin-embedded tissue blocks were selected for immunohistochemistry, which was performed using commercially available antibodies at the University of Michigan according to standard protocols. When available, immunohistochemistry previously performed during the initial diagnosis of each case was reviewed.

A panel including pan-cytokeratin (CK) cocktail [AE1/3 (Chemicon International, San Diego, CA, USA; 1:800), cytokeratin 7 (CK7) (Dako; 1:125, 30 min), cytokeratin 20 (CK20) (Dako; 1:125, 30 min), carbonic anhydrase IX (CA IX) (Novus Biologicals, Littleton, CO, USA; 1:200, 30 min), CD117 (Dako, 1:600), a-methylacyl-CoA racemase (AMACR; P504s) (Zeta Corporation, Tuscon, AZ, USA; 1:20, 30 min), SDHB, Fumarate Hydratase (FH), HMB45 (Dako; 1:25, 30 min) and Melan-A (Dako; 1:50, 30 min), was employed as necessary.

Cytogenetic Analysis

Fluorescent in situ hybridization (FISH) for TFE3 and/or TFEB gene rearrangements were performed on representative formalin fixed paraffin embedded (FFPE) tumor tissue from cases that were morphologically suspicious for MiT family translocation renal cell carcinoma using our in-house Dual-color break-apart clinical TFE3 and TFEB FISH assay using custom-made probes (Empire Genomics, Buffalo, NY), according to previously described procedure protocol [12].

Results

A total of 68 cases with confirmed diagnosis of RCC at 30 years of age or younger were identified and included in our study. The demographics and clinicopathological features of these patients and their tumors are detailed in Tables 1-2 and Supplementary Table 1. The median age at diagnosis was 25.5 years (mean=23, range 7 to 30 years). The cohort consisted of 50% females (n=34/68) and 50% males (n=34/68). Sixty-three percent of the patients (n=43/68) underwent partial resection whereas 37% (n=25/68) had radical nephrectomy. The median tumor size at the time of surgical resection was 3.7 cm (mean=5.3, range 1.0 to 30.0 cm). The majority of the tumors presented as a single focus (83%, n=57/68) while 17% of the tumors (n=11/68) were multifocal.

CCRCC was the most common histologic subtype, with non-VHL and VHL associated RCCs making up 35% (n=23/68) and 18% (n=12/68) of the entire cohort, respectively. Other tumors seen in this cohort were MiT family translocation RCC (13%, n=9/68), RCC unclassified (12%, n=8/68), Type 1 PRCC (7%, n=5/68), TSC- associated RCC (6%, n=4/68), ChRCC (4%, n=3/68), SDH –deficient RCC (3%, n=2/68), Type 2 PRCC (1%, n=1/68) and Renal medullary carcinoma (1%, n=1/68). Clear cell papillary RCC, HLRCC-associated RCC, Tubulocystic RCC and ACD-RCC were not identified in our current cohort.

The World Health Organization/International Society of Urological Pathology (WHO/ISUP) grading system was applied to CCRCC and PRCC. Of those tumors (n=41), 71% (n=29/41) were

grade 2, 27% (n=11/41) were grade 3 and 2% (n=1/41) were grade 4; while CCRCC were more commonly WHO/ISUP grade 2, PRCC was slightly enriched in higher grade cases (Table 2 and Supplementary Table 1). History of end-stage renal disease (ESRD) was documented in 3% (n=2/68) of the patients, one patient had VHL associated CCRCC and the other had TSC-associated RCC as their tumor subtype. Sixty-three percent of the patients presented with clinical stage 1 disease (n=43/68), 15% stage 2 (n=10/68), 15% stage 3 (n=10/68) and 4% (n=3/68) stage 4. Three percent of the cases had unknown clinical stage (n=2/68). Advanced stage disease (stages 3 and 4) were more commonly seen in type 2 PRCC (100%, n=1/1) and renal medullary carcinoma (100%, n=1/1), followed by MiT family translocation RCC (78%, n=7/9), type 1 PRCC (40%, n=2/5) and RCC unclassified (25%, n=2/8).

A large portion of the cohort were patients with a familial kidney cancer syndrome (26%, n=18/68); VHL syndrome associated RCC accounted for 18% (n=12/68), TSC was associated with 6% of RCC (n=4/68), while SDH-deficient RCC accounted for 3% (n=2/68) of the entire cohort. All VHL patients demonstrated renal tumors with CCRCC morphology and all TSC patients had TSC-associated RCC.

Three patients (4%) had a prior history of chemotherapy (see supplementary Table 1) for previous histories of Wilms tumor (Patient 1), hepatoblastoma (Patient 6) and Neuroblastoma (Patient 25). Of these patients, two developed MiT family translocation RCC (with TFE3 gene rearrangement) and one had CCRCC.

Clear Cell Renal Cell Carcinoma

CCRCC was the most common RCC (53%, n=35/68) in our cohort (Table 1), with non-VHL associated CCRCC making up 35% (n=23/68) of the cohort and VHL-associated CCRCC 18% (n=12/68). The median age for non-VHL RCC patients was 27 years (mean=25.8, range 20 to 30 years), while the median age for VHL associated RCC patients was 27 years (mean=26.9, range 20 to 30 years. The non-VHL CCRCC showed classic histology (Figure 1A and 1B). All non-VHL CCRCC tumors were unifocal.

VHL associated CCRCC often showed classic CCRCC histology similar to sporadic cases (Figure 2A). Many cases (n=8/12) however, showed cystic changes (Figures 4A and 4B), a

feature possibly enriched in VHL associated CCRCC. Immunohistochemical assessment of one of these tumors demonstrated patchy CK7 expression (Figure 4C), with a strong/complete membranous CAIX staining (Figure 4D) and cytokeratin AE1/AE3 staining, supportive of the diagnosis of CCRCC. Seventy-five percent (n=9/12) of VHL associated CCRCC were multifocal. Germline VHL mutations/rearrangements were confirmed in 75% (n=9/12) of VHL patients in the cohort (Supplementary Table 1); history was provided for the remaining 3 patients.

MiT Family Translocation Renal Cell Carcinoma

Nine of the 68 tumors (13%) were classified as MiT family translocation RCC based on the presence of previously described morphologic, immunophenotypic and cytogenetic features. The median age at presentation was 18 years (mean=18.1, range 7 to 30 years). Seven were transcription factor E3 (TFE3) translocated RCC while 2 were transcription factor EB (TFEB) translocated RCC. TFE3 translocated tumors showed mixed papillary and clear cell features, high-grade nuclei and areas with classic voluminous cytoplasm and bulging distinct cell borders, reminiscent of soap bubbles as previously described (Figure 3A and B). Other architectural patterns including solid sheets, trabeculae, pseudopapillae were also seen. A few of these tumors contained occasional psammoma bodies. Break apart FISH probe confirmed the presence of TFE3 translocation in all cases (Figure 3C). TFEB translocated tumors exhibited a characteristic biphasic cell population, consisting of large cells with eosinophilic and granular-to-clear cytoplasm and vesicular nuclei with prominent nucleoli admixed with small cells with scant cytoplasm and dense chromatin (Figure 3D and E). These tumors exhibited diffuse expression of at least 1 marker of melanocytic differentiation (Melan-A or HMB45) (figures 5C and D). Break apart FISH probe confirmed the presence of TFEB translocation in all cases (Figure 3F). No TFEB amplified tumors were seen in this cohort thus reflecting that this newly described tumor entity [12, 17] was not seen in this younger cohort of patients.

Papillary Renal Cell Carcinoma

Six tumors were classified as PRCC based on morphology and immunophenotype;

Type 1 and type 2 PRCC comprised 7% (5/68) and 1% (1/68) of the cohort, respectively. The median age at diagnosis was 22 years (mean=22.5 and range 16 to 30 years). Type 1 and Type 2 tumors showed classical histologic features (Figure 1C). Immunohistochemical staining showed positive CK7 and AMACR expression in all tumors.

Chromophobe Renal Cell Carcinoma

Three cases of ChRCC were identified in our cohort. The median age at diagnosis was 23 years (mean=24, range 19 to 29 years). All tumors demonstrated classic features (Figure 1D). No cases of eosinophilic variant of ChRCC were recognized in this cohort. All tumors demonstrated diffuse CD117 and CK7 expression by immunohistochemistry.

Renal Medullary Carcinoma

One renal tumor (1%) in this cohort was consistent with renal medullary carcinoma, diagnosed in a patient with a documented history of sickle cell trait. Histologically, the tumor had classic morphologic features including cells with eosinophilic cytoplasm and high-nuclear grade arranged in tubular and reticular structures with an infiltrative growth pattern, desomoplastic stroma and neutrophilic infiltrate (Figure 1E).

Renal Cell Carcinoma Associated with Familial Cancer Syndromes

Familial cancer syndromes made up 26% (n=18/68) of our cohort. VHL associated RCC were previously discussed in the CCRCC section. TSC was associated with 6% (n=4/68) of RCC in this cohort. The median age at diagnosis was 15 years (mean= 16, range 7 to 26 years). Three of the four cases demonstrated granular-eosinophilic macrocystic pattern with papillary architecture (Figure 2C) or nested architecture surrounded by delicate vasculature (Figure 2D). One case demonstrated renal angiomyoadenomatous-like histology (Figure 2B) with nested and cystic clear cells and thick fibromuscular septa. Two tumors were multifocal.

Two cases (3%) had succinate dehydrogenase deficiency syndrome with an associated SDHB-deficient RCC. The patients had a median age of 21 years (mean=21, range 13 to 29 years). Histologically, these tumors were composed of granular, eosinophilic cells arranged in a nested pattern, with characteristic flocculent cytoplasm and eosinophilic inclusions (Figure 2E and 5A). SDHB IHC demonstrating loss of SDHB (Figure 5B) supportive of the diagnosis. All cases demonstrated intact fumarate hydratase (FH) staining.

Renal cell carcinoma, unclassified

Eight tumors (12%) could not be placed into the well-established renal tumor entities according to the new WHO 2016 classification, and hence were placed in the RCC, unclassified category (Figures 1F and 2F). The median age at diagnosis for these patients was 23 years (mean=22.5, range 16 to 30 years). Most tumors in our cohort demonstrated features of high-grade tumors with variable architectures (rhabdoid, alveolar, papillary, tubulopapillary, glandular or sarcomatoid architecture) with significant cytologic atypia. These tumors could not be classified based on their unusual histologic features coupled with negative or variable immunohistochemical and cytogenetic profile. Two tumors had the characteristic morphologic features of MiT family translocation RCC, however evidence of TFE3/TFEB gene rearrangements could not be documented by FISH evaluation. One tumor was suspicious for SDH-deficient RCC because of its eosinophilic morphology, however SDHB expression by IHC was intact. Eosinophilic solid and cystic solid RCC [18] were excluded based upon a lack of classic morphologic features coupled with negative CK 20 expression by immunohistochemistry. All cases were seen to exhibit high WHO/ISUP grade (3 or higher).

Follow up data

The median follow-up period was 3 years (range 11 days-28.4 years) (Table 2). During the follow-up interval, 7% (n=5/68) experienced metastasis and 9% (n=6/68) died of the disease. For patient who died of the disease (Table 3), MiT family translocation RCC was the most common histologic diagnosis (68%, n=4/6). Additionally, 50% (n=3/6) of the patients who died of the disease had developed recurrence/metastasis and 83% (n=5/6) had advanced stage disease.

Discussion

In children and young adults, RCC is relatively less common than in adults. The morphologic classification of adult RCC according to the WHO 2016 criteria has been supported by the detection of specific genetic alterations that distinguish RCC and their clinical course. In contrast, the rarer RCC in young adults have only recently begun to be better characterized. We have investigated 68 RCC in children and young adults (≤ 30 years), comprising all RCC from the University of Michigan health system between 1986 and 2018. Speculation remains whether RCC in younger patients represents a truly different entity from its adult counterpart [3-5]. Our results suggest that RCCs in this age group share many similarities to those in adults, yet possess some distinct clinicopathological characteristics. A gender predominance has not been reported for RCC in children [19], while in adults, a male predominance has been reported (male: female ratio 2:1) [20]; male: female ratio in our series was 1:1.

In this study, we sought to explore the applicability of the new 2016 WHO classification to our set of RCCs. We found that CCRCC was the most common subtype in this age group, similar to what has been reported in the adult population, however at a lower percentage. In several studies of CCRCC in young adults [19, 21, 22], CCRCC was reported at frequencies ranging from 6% up to 73%. Our series showed a frequency of 53% which is similar to what was reported by Lopez et al. (50.7%) [23]. Most of the tumors had classic clear cell morphology and had low-grade nuclei. VHL associated CCRCC were commonly found in this cohort in accordance with previous studies [13] and were also seen to frequently demonstrate cystic changes and multifocality. None of the VHL associated CCRCC in this cohort were associated with disease specific mortality which reflects the relative better prognosis associated with such patients.

MiT family translocation RCC has been initially recognized in the 2004 WHO classification as a distinct molecular subtype of RCC with a predilection for pediatric patients, although they are now increasingly being reported in adults [12, 24, 25]. This subtype represents a significant proportion of RCC in pediatric patients, with widely divergent frequencies reported in recent series (20-75%) [26, 27]. In our series, MiT family translocation RCC was the second most

common subtype, accounting for 13% of our cohort, confirmed by the presence of TFE3/TFEB gene rearrangements. The biological behavior of MiT family translocation RCC in children and young adults is controversial. Whereas some series have suggested a good prognosis, others report that prognosis is dependent on the overall stage of tumor and completeness of resection similar to that seen in adult patients [28]. In our series, the majority (seven of nine) of MiT family translocation RCC presented at an advanced stage disease. The majority also had poor outcomes, with distant metastasis and/or death often occurring years after initial presentation. All MiT family translocation RCC in our cohort that died of the disease had TFE3 gene rearrangement. Similarly, Xu et al. [29] found that compared with non-Xp11.2 translocation RCCs, Xp11.2 translocation RCCs showed a higher tumor grade, advanced pathologic stage and poorer cancer-specific survival rates in young adults. Furthermore, all the MIT family translocation RCC in this cohort were related to translocation of TFE3 and TFEB genes, and none of them demonstrated presence of TFEB amplification, which supports the previous observations that this newly described entity is relatively enriched in the older age group patients [12, 17].

Although several previous series of pediatric RCC do not mention any unusual predisposing conditions or underlying syndromes [30, 31], other series [32] report approximately 20-30% of patients with underlying predisposing conditions or syndromes, such as history of chemotherapy, neuroblastoma, renal failure or tuberous sclerosis. Similarly, in our series, RCC associated with familial cancer syndrome or hereditary renal cell carcinoma syndromes accounted for 26% of the cases. Although the reported age of presentation of VHL associated RCC is much younger than that reported in sporadic RCC (mean age of onset 37 vs 61 years), in the majority of the previously published pediatric series [4, 32, 33], VHL associated RCC has not been reported. The median age of VHL associated RCC patients in our series was 27 years, again confirming that patients with VHL syndromes present with RCC at much younger ages. In addition, we identified SDH-deficient RCC, a subtype that was recently formally recognized in the 2016 WHO classification system, in two patients, who were both found to be SDHB deficient. SDH-deficient RCC occurs in patients with germline mutations in one of the SDH subunit genes, although SDHB mutation is the most common. Loss of immunohistochemical labeling in SDH-deficient RCC (similar to what is seen in our study) reflects the resulting destabilization of the

SDH complex. Histologically, the most distinctive feature is the presence of cytoplasmic vacuoles and inclusion-like spaces containing eosinophilic fluid or flocculent material, which was seen in both of our cases. Furthermore, we identified 4 cases of RCC developing in the setting of TSC. TSC-associated RCC is known to demonstrate significant heterogeneity with 3 main histologic patterns [15]. Three of our TSC- associated tumors exhibited a granular eosinophilic-macrocystic histology similar to what was previously described [15] [34] while one tumor resembled renal angioadenomatous tumor (RAT-like) or RCC with smooth muscle stroma.

Furthermore, three patients had a history of previous exposure to chemotherapy for Wilms tumor (1 patient), hepatoblastoma (1 patient) and neuroblastoma (1 patient). Of these patients, two developed MiT family translocation RCC while the third developed CCRCC. These findings support the reported association of approximately 10-15% of translocation RCC with prior exposure to chemotherapy [35-39]. Argani et al. [37] described MiT family translocation RCC that arose in young patients who had received chemotherapy. Histologically, these tumors showed typical features described for translocation RCCs. The interval between chemotherapy and the diagnosis of RCC ranged from 4 to 13 years. The apparent predilection of these tumors for children raises the possibility that the relatively increased proliferation that occurs in the growing pediatric kidney may render it more sensitive to the mutagenic effects of several chemotherapies. Falzarno et al. [39] also described a higher risk of RCC in younger patients who are survivors of childhood cancers, particularly neuroblastoma. In their study, they observed that in addition to the carcinogenic effects of chemotherapy and or radiation therapy, predisposing underlying genetic conditions and individual susceptibility to therapy side effects may also play a role in the pathogenesis of second malignancies. Almost half of the tumors included in their study showed morphologic features of MiT family translocation RCC while the remaining half showed oncocytic cells arranged in tubular and solid architecture. Among RCCs, translocation RCCs have been strongly associated to exposure to cytotoxic chemotherapy including alkylating agents like cyclophosphamide and DNA topoisomerase II inhibitors.

Despite applying the new 2016 classification, a high proportion of RCC (12%) in our series remained unclassified. These tumors had a wide variety of architectural pattern, high-grade nuclear features and often eosinophilic morphology. FH and SDHB by IHC in addition to a panel

of immunostains as well as cytogenetic studies were inconclusive. A recent study by Li et al. [40], reclassified 22 out of 33 unclassified RCC in children and young adults that were characterized by predominantly eosinophilic cytoplasm to either SDH-deficient RCC, FH-deficient RCC (HLRCC) or eosinophilic solid and cystic RCC, however 11 cases (33%) remained unclassified. Pathologists should therefore have a low threshold for performing FH and SDHB IHC when confronted with unclassified eosinophilic RCC in young patients. However, it should be noted that although a renal tumor with FH negative immunophenotype carries a strong correlation with the presence of the FH mutation at the germline level, the type of FH mutation itself may determine whether FH protein loss can be detected by immunohistochemical evaluation. A small subset of patients with HLRCC-associated RCC may demonstrate equivocal results or retain FH expression within the tumor; a correlated finding reported in the literature is that tumors from patients with FH missense mutations may show equivocal or retained FH expression [41].

Additionally, two tumors that we reported as unclassified RCC had the characteristic morphologic findings of MiT family translocation RCC, however evidence of TFE3/TFEB gene rearrangements could not be confirmed by FISH evaluation. While break-apart FISH assays for TFE3 and TFEB avoid issues related to PCR amplifications and is easier to conduct than RT-PCR, false- negative results using common break-apart FISH probes in Xp11 translocation RCC with rare fusions such as RBM10-TFE3 or NONO-TFE3 fusions has been reported and should be considered [42].

Finally, Clear cell papillary RCC, recognized as a distinct entity in the new 2016 WHO classification, was not seen in our cohort, however this entity has been previously described in young adults. Lopez et al. [23] have reported that 9.3% of their RCC series in patients 40 years old or younger were of the clear cell papillary histology.

Although we note the limitations of our study, namely a retrospective review, it is one of the largest series to date addressing the spectrum of RCCs in children and young adults according the recent 2016 WHO classification of renal tumors.

In summary, we describe the morphologic, immunophenotypic and molecular spectrum of RCC in patients 30 years of age or younger. Our results suggest that RCC in children and young adults is a relatively uncommon disease that shares many histologic similarities to RCC occurring in adults and yet demonstrate some unique clinicopathological differences. MiT family translocation RCC and rare familial syndrome subtypes are relatively more frequent in the pediatric and adolescent age groups than in adults. CCRCC is less frequent than in adults, however accounted for the most common subtype seen in this age group. MiT family translocation RCC patients presented with advanced stage disease and had poor clinical outcomes. The large and heterogeneous subgroup of unclassified RCC contains phenotypically distinct tumors with further potential for future subcategories in the renal cell carcinoma classification.

Figure Legends

Figure 1:
RCC subtypes in our cohort of patients 30 years of age or younger including (A and B) CCRCC, (C) papillary RCC (PRCC), (D) chromophobe RCC, and (E) renal medullary carcinoma. In addition, a subset of patients had tumors that could not be further subclassified based on current WHO diagnostic criteria; these tumors were designated RCC, unclassified (F). Panel insets show higher magnification (X400) to highlight microscopic morphology features.

Figure 2:
Syndrome-associated RCC subtypes in patients 30 years of age or younger. In addition to conventional RCC subtypes, a number of patients had syndrome- associated RCC subtypes, including: (A) VHL-associated CCRCC; (B) TSC-associated carcinomas resembling renal angiomyoadenomatous tumor (RAT-like) or RCC with smooth muscle stroma; (C,D) TSC-associated carcinomas with a granular eosinophilic histology; (E) SDH-deficient RCC with flocculent eosinophilic inclusions and, (F) an RCC, unclassified, with oncocytic/morphologic features suggestive of FH or SDHB deficiency, however, this patient did not have any germ line pathogenic mutation.

Figure 3:
MiT family translocation RCC in patients 30 years of age or younger. (A, B) H&E images demonstrating mixed papillary and clear cell features in two cases of TFE3 translocated RCC. Although morphologically suspicious for translocation RCC, these cases were initially designated as RCC, unclassified. Subsequent FISH (C) demonstrated TFE3 gene rearrangements in both cases, confirming the diagnosis. (D, E) H&E images showing the classic “biphasic” appearance in a case of TFEB translocated RCC. Based on morphology alone, this case was

highly suspicious for TFEB translocated RCC, and subsequent FISH (F) confirmed a TFEB gene rearrangement. For the FISH images in (C) and (F), yellow = fusion (wild type) and red/green = break-apart (rearranged).

Figure 4:
H&E images showing cystic CCRCC (A and B) with positive CK7 (C) and CAIX (D) immunostains.

Figure 5:
H&E images showing SDH-deficient RCC (A) with confirmatory immunostain demonstrating loss of SDHB (B). H&E images showing the classic "biphasic" appearance in a case of TFEB translocated RCC (C) showing diffuse melan-A staining (D).

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Table 1: Renal Cell Carcinoma Histologic Subtypes in our patient cohort

	Number	Percentage
Tumor Type		
CCRCC (non-VHL)	23	35%
CCRCC (VHL-associated)	12	18%
tRCC	9	13%
RCC, unclassfied	8	12%
Type 1 PRCC	5	7%
TSC-associated RCC	4	6%
ChRCC	3	4%
SDH-deficient RCC	2	3%
Type 2 PRCC	1	1%
Renal medullary carcinoma	1	1%

CCRCC = clear cell renal cell carcinoma; tRCC = translocation-associated renal cell carcinoma; PRCC = papillary renal cell carcinoma; ChRCC = chromophobe renal cell carcinoma; RCC = renal cell carcinoma; VHL = Von-Hippel Lindau; SDH = succinate dehydrogenase, TSC = tuberous sclerosis complex

Table 2: Clinical, pathological and follow up data for our RCC cohort

	Number	Percentage
WHO/ISUP grade*		
1	0	0%
2	29	71%
3	11	27%
4	1	2%
ESRD		
Yes	2	3%
No	66	97%
Pathologic T stage		
T1a	36	53%
T1b	11	16%
T2a	4	6%
T2b	8	12%
T3a or greater	5	7%
Unknown	4	6%
Pathologic N stage		

N0 or NX	56	82%
N1	8	12%
Unknown	4	6%
Pathologic M stage		
M0 or MX	63	93%
M1	3	4%
Unknown	2	3%
Clinical stage		
1	43	63%
2	10	15%
3	10	15%
4	3	4%
Unknown	2	3%
Follow-up		
Mean	5.4 years	
Median	3.0 years	
Range	11 days - 28.4 years	
Subsequent metastases		
Yes	5	7%
No	59	87%
Not applicable or unknown	4	6%
Dead of disease		

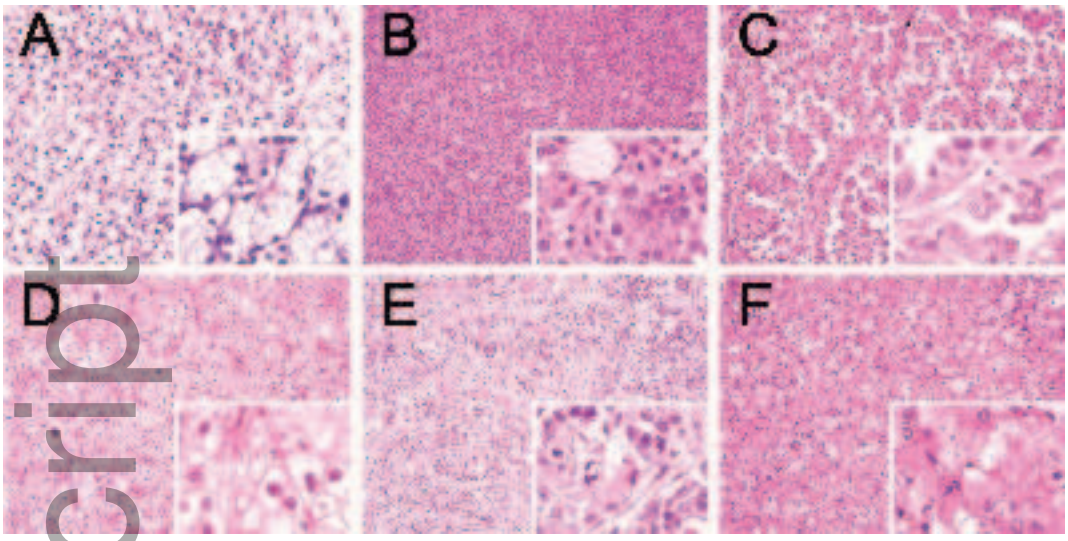
Yes	6	9%
No	61	90%
Unknown	1	1%

***WHO/ISUP grading applicable to clear cell RCC and papillary RCC only**

Table 3: Clinicopathologic data for RCC patients who died of disease

	Number	Percentage
Age		
Median (years)	15	
Gender		
M	3	50%
F	3	50%
Histologic Type		
tRCC	4	68%
Medullary RCC	1	16%
RCC, unclassified	1	16%
WHO/ISUP grade		
1	0	0%
2	0	0%
3	4	68%
4	2	32%
VHL		

Yes	0	0%
No	6	100%
Metastasis		
Yes	3	50%
No	3	50%
Clinical Stage		
1	1	17%
2	0	0%
3	2	33%
4	3	50%



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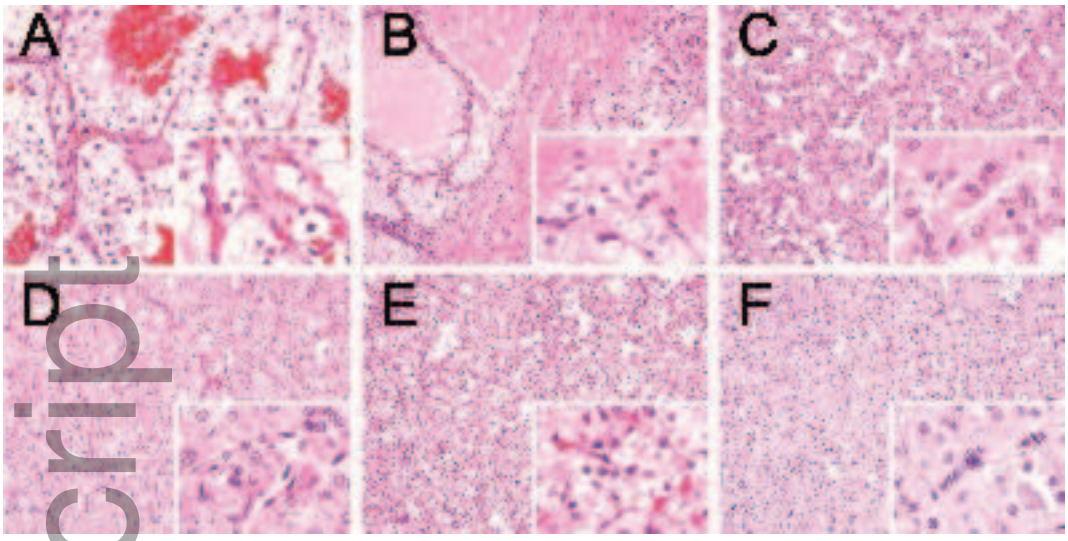

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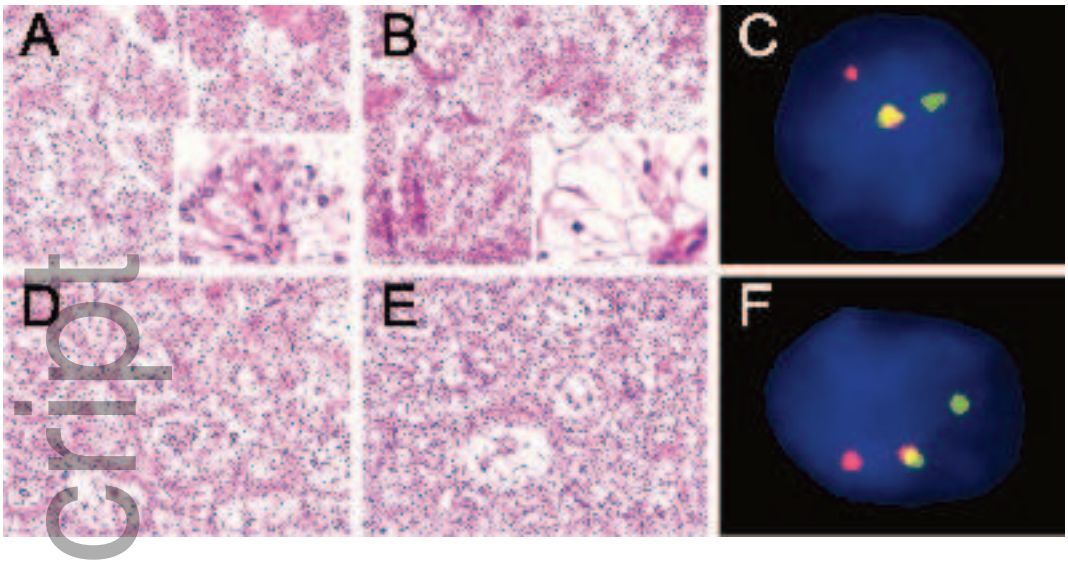

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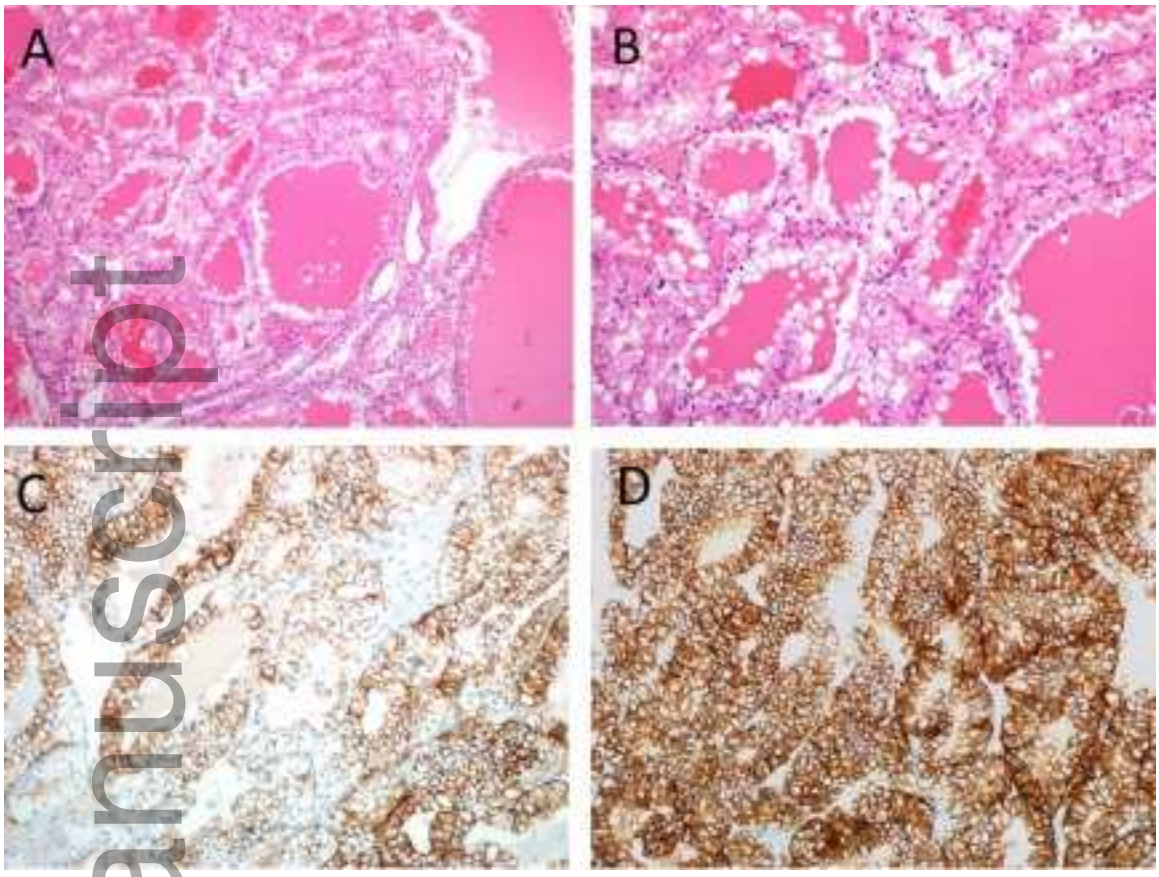

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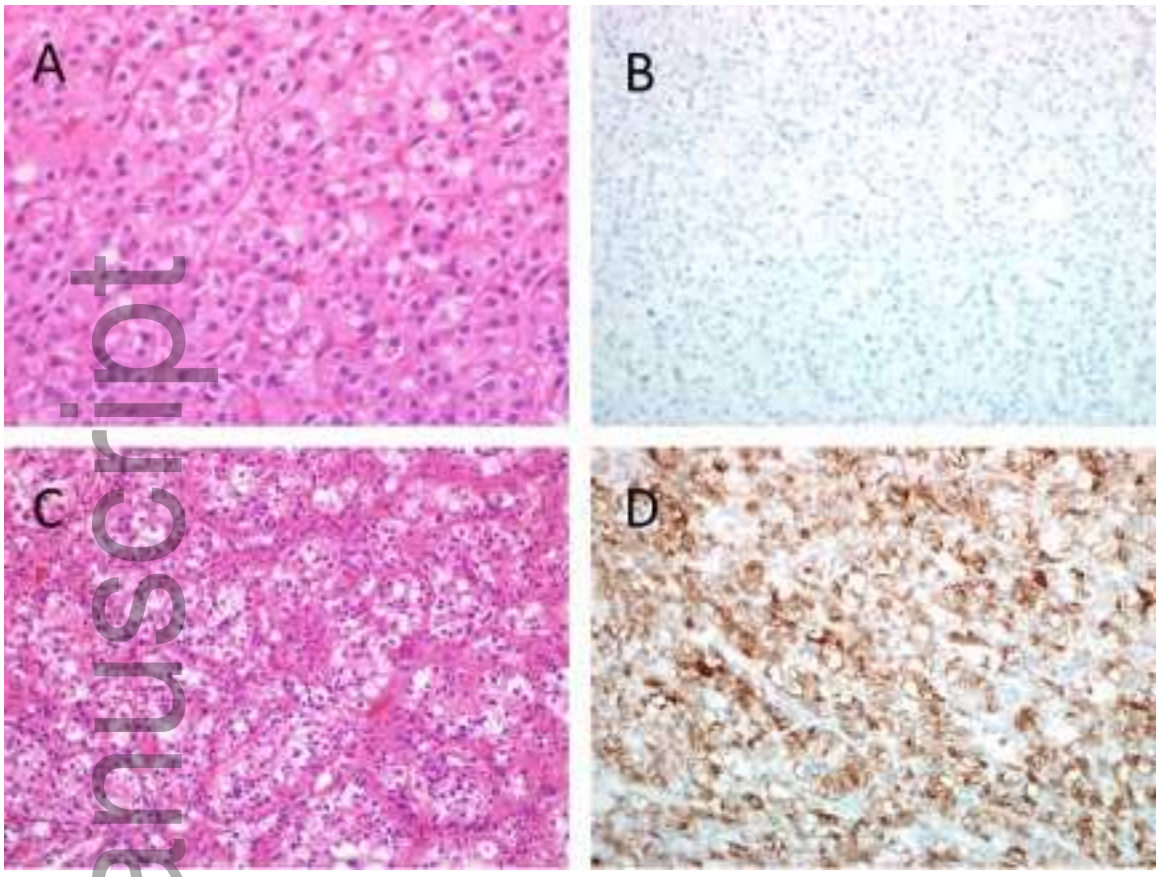

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