The Classification of Pediatric and Young Adult Renal Cell Carcinomas Registered on the Children’s Oncology Group (COG) Protocol AREN03B2 After Focused Genetic Testing

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BACKGROUND: Renal cell carcinomas (RCCs) are rare in young patients. Knowledge of their pathologic and molecular spectrum remains limited, and no prospective studies have been performed to date in this population. This study analyzes patients diagnosed with RCC who were prospectively enrolled in the AREN03B2 Children’s Oncology Group (COG). The objective was to classify these tumors with the aid of focused genetic testing and to characterize their features. METHODS: All tumors registered as RCC by central review were retrospectively re-reviewed and underwent additional ancillary studies. Tumors were classified according to the 2016 World Health Organization classification system when possible. RESULTS: In total, 212 tumors were identified, and these were classified as microphthalmia transcription factor (MiT) translocation RCC (MiT-RCC) (41.5%), papillary RCC (16.5%), renal medullary carcinoma (12.3%), chromophobe RCC (6.6%), clear cell RCC (3.3%), fumarate hydratase-deficient RCC (1.4%), and succinate dehydrogenase-deficient RCC (0.5%). Other subtypes included tuberous sclerosis-associated RCC (4.2%), anaplastic lymphoma kinase (ALK)-rearranged RCC (3.8%), thyroid-like RCC (1.4%), myoepithelial carcinoma (0.9%), and unclassified (7.5%). MiT-RCCs were classified as either transcription factor E3 (TFE3) (93.2%) or EB (TFEB) (6.8%) translocations, and characterization of fusion partners was possible in most tumors. CONCLUSIONS: The current study delineates the frequency of distinct RCC subtypes in a large prospective series of young patients and contributes knowledge to the diagnostic, clinical, and genetic features of MiT-RCC, the most common subtype among this age group. The identification of rare subtypes expands the spectrum of RCC in young patients, supporting the need for a thorough diagnostic workup. These studies may aid in the introduction of specific therapies for different RCC subtypes in the future. Cancer 2018;124:3381-9, © 2018 American Cancer Society.

KEYWORDS: pediatric renal tumor, renal cell carcinoma, transcription factor E3 (TFE3) gene, transcription factor EB (TFEB) gene, translocation renal cell carcinoma.

INTRODUCTION
Renal cell carcinomas (RCCs) account for only 2% to 4% of pediatric renal tumors and differ morphologically and genetically from their adult counterparts. Although the majority of adult RCCs are clear cell RCC (CCRCC) (range, 65%-70%), those are quite rare in the pediatric age group. In contrast, RCCs with translocations involving members of the microphthalmia transcription factor (MiT) family (transcription factor E3 [TFE3] and transcription factor EB [TFEB]) occur more frequently in younger patients. Historically, approximately 16% to 24% of pediatric RCCs remain unclassified, preventing clear diagnostic and therapeutic strategies. Before the Children’s Oncology Group (COG) AREN03B2 protocol, no prospective studies had been performed on pediatric RCCs, and knowledge of their pathologic and molecular spectra, biologic features, and clinical behavior remains limited. To address this, our objective was to analyze patients diagnosed with RCC who were enrolled in the AREN03B2 COG protocol and to apply focused genetic testing to clarify their classification. To our knowledge, this represents the first comprehensive analysis of unselected RCCs in young patients registered prospectively on a clinical study.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

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MATERIALS AND METHODS

Clinical Specimens
All patients enrolled in the AREN03B2 protocol between August 2006 and July 2016 who were diagnosed with RCC by central pathology review were included in this study. Approval from the National Institutes of Health Central Institutional Review Board and subsequently from the local Institutional Review Board for the collection of biologic material and clinical data was obtained before enrollment. Patients were enrolled from participating COG institutions in the United States, Canada, and Australia. Eligibility criteria included the first occurrence of a kidney tumor in a patient younger than 30 years. A full set of hematoxylin and eosin slides, the institutional pathology report, and available immunohistochemical, cytogenetic, and molecular analyses performed at the originating institutions underwent central pathology review, and tumors were classified as MiT translocation-RCC (MiT-RCC), papillary RCC (PRCC), renal medullary carcinoma (RMC), chromophobe RCC, CCRCC, or RCC-not otherwise specified (RCC-NOS). All cases were also reviewed centrally by an expert panel of surgeons, oncologists, and radiologists for central assignment of TMN staging. AREN03B2 is a classification protocol that does not include therapeutic recommendations.

For the current study, all cases were retrospectively reviewed by 3 pathologists (M.M.C., S.M.R., and E.J.P.); and, in all cases, consensus was achieved. Additional immunohistochemistry, interphase fluorescence in situ hybridization (FISH) or next-generation sequencing (NGS) analysis was performed on available archival formalin-fixed, paraffin-embedded tissues or touch preparations using a focused strategy based on the histologic appearance, as described below. Tumors were classified into distinct RCC subtypes according to the 2016 World Health Organization classification system when possible.\(^4\)

### Diagnosis of MiT-RCC
Given a histologic appearance consistent with the diagnosis of MiT-RCC,\(^4,9\) a prioritized sequence of analyses was performed to efficiently use available (and often limited) tissue, including (in order of priority): 1) TFE3 immunohistochemistry, 2) TFE3 and/or TFEB break-apart FISH, 3) PRCC translocation-associated gene (PRCC)/TFE3 and alveolar soft part sarcoma locus (ASPL)/TFE3 dual-color fusion probe set FISH, and 4) NGS for TFE3 fusion transcripts, as described in the Supporting Methods.

### Diagnosis of PRCC, RMC, Chromophobe RCC, and CCRCC
Given a histologic appearance consistent with the above RCC subtypes recognized by the latest World Health Organization classification, the appropriate immunohistochemical analyses were performed for confirmation, as described below.

### Diagnosis of Tumors Classified as RCC-NOS
If the histologic appearance and immunohistochemical profile were atypical or unusual, precluding confident classification into the RCC subtypes described above,
then tumors were initially classified as RCC-NOS. NGS for mutations in 11 genes known to be recurrently mutated in RCC, as described in the Supporting Methods, was performed in a subset of tumors for which archival tissue was available. In selected cases (as described below; see Results), analysis for unusual fusion transcripts was also performed using the Archer kit (Enzymatics, Beverly, MA) as described in the Supporting Methods. \(^1^0\)

**RESULTS**

The classification after retrospective pathologic review and focused genetic analysis with clinical demographics and staging of 212 RCCs are summarized in Table 1. Results of the focused molecular analysis are summarized in Table 2. After central pathology review, 42 of 212 tumors (19.8%) were initially classified as RCC-NOS; among those, 26 of 42 tumors (61.9%) could be reclassified after the focused genetic analysis. A brief description of each category is provided below.

**MIT-RCC**

Eighty-eight of 212 tumors (41.5%) were classified as MiT-RCC based on the presence of previously described classic morphologic and immunophenotypic features\(^4^,^9\): 82 of 88 (93.2%) were MiT-RCC with TFE3 translocation, and 6 of 88 (6.8%) were MiT-RCC with TFE3 translocation, as described below. No differences were noted in TNM staging when comparing tumors from patients ages 0 to 0 with those from patients ages 11 to 20 years (stage I, 19.4% vs 23.3%, respectively; stage II, 6.5% vs 1.3%, respectively; stage III, 58% vs 64%, respectively; stage IV, 16.1% vs 11.7%, respectively).

**TFE3 MIT-RCC**

Eighty-two tumors had diffuse, strong nuclear immunohistochemical expression of TFE3 and/or genetic confirmation of a TFE3 gene rearrangement. Immunohistochemical TFE3 expression was observed in all 78 tumors that were available for testing (Fig. 1A). Further evidence of TFE3 gene rearrangement was present in 72 of 82 tumors, including all 5 in which TFE3 immunohistochemistry was not performed. The specific fusion partner was identified in 56 of 72 tumors by either dual-probe FISH or NGS (Table 2). The partner was ASPL-TFE3 (25), PRCC-TFE3 (24), SFPQ-TFE3 (5), NONO-TFE3 (1), MED15-TFE3 (1), ?-TFE3 (16)*, and ?-TFEB (6)*.  

**TABLE 2. Genetic Abnormalities Identified in 100 Renal Cell Carcinomas**

| Diagnosis                           | Genetic Abnormality on Tumor (No. Positive) |
|-------------------------------------|---------------------------------------------|
| MIT-RCC                             | ASPL-TFE3 (25)                              |
|                                     | PRCC-TFE3 (24)                              |
|                                     | SFPQ-TFE3 (5)                               |
|                                     | NONO-TFE3 (1)                               |
|                                     | MED15-TFE3 (1)                              |
|                                     | ?-TFE3 (16)*                                |
|                                     | ?-TFEB (6)*                                 |
| Papillary RCC                       | Trisomy 7 and 17 (3)                        |
| Clear cell RCC                     | t(3;5)(p13;q31) (1)                         |
| Fumarate hydratase-deficient RCC    | FH exon6.c.839G>A + LOH (1)                 |
|                                    | FH exon7.c.914T>C + LOH (1)                 |
|                                    | FH exon3.c.301C>T + LOH (1)                 |
| Succinate dehydrogenase-deficient RCC | SDHB [germline mutation, details not available] (1) |
| ALK-rearranged RCC                  | VCL-ALK (3)                                 |
|                                    | TPM3-ALK (3)                                |
|                                    | HOOK1-ALK (1)                               |
|                                    | STRN-ALK (1)                                |
| Tuberous sclerosis-associated RCC   | TSC2 exon8.c.709.710insCCTGTTTGATCTGTTCCCTCTGTCGCCACCAAT (1) |
|                                    | TSC2 exon27.c.3061G>T + exon8.c.669dupC (1) |
|                                    | TSC2 exon25.c.2833A>T + exon211.c.2344A>G>T (1) |
|                                    | TSC2 exon24.c.2714G>A + LOH (1)             |
| Myoepithelial carcinoma             | KLF15-EWSR1 (2)                             |

Abbreviations: ALK, anaplastic lymphoma kinase; ASPL, alveolar soft part sarcoma locus; dup, duplication; EWSR1, Ewing sarcoma breakpoint region 1; FH, fumarate hydratase; HOOK1, hook homolog 1; ins, insertion; KLF15, Kruppel-like factor 15; LOH, loss of heterozygosity; MED15, mediator complex subunit 15; MIT-RCC, renal cell carcinoma with translocations involving members of the microphthalmia transcription factor (MiT); NONO, non-POU domain-containing octamer binding protein; PRCC, papillary renal carcinoma translocation-associated gene; RCC, renal cell carcinoma; SDHB, succinate dehydrogenase complex, subunit B; SFPQ, splicing factor, proline-rich and glutamine rich; STRN, striatin, calmodulin-binding protein factor; t, translocation; TFE3, transcription factor E3; TFE6, transcription factor EB; TPM3, tropomyosin 3; TSC2, tuberous sclerosis protein 2; VCL, vinculin.  
*This gene rearrangement involves an unknown partner (?) and either TFE3 or TFE6.
complex subunit 15 (MED15) (22q11) in 1 tumor. Evidence of TFE3 gene rearrangement was not demonstrated in 10 tumors that were identified as TFE3-positive by immunohistochemistry. Two tumors exhibited normal signals by FISH, indicating that the assay worked but was negative for the translocation; whereas, in 8 tumors, the assay either failed or insufficient material precluded testing.

Two cytologic patterns exhibiting some overlap were appreciated, similar to previous observations.9 Pattern 1 (42 tumors) corresponded to cells with voluminous cytoplasm and well defined cell membranes (Fig. 1B). Pattern 2 (40 tumors) consisted of smaller cells with less distinct cell membranes (Fig. 1C). Most tumors (21 of 25) with ASPL-TFE3 fusion transcripts had pattern 1, whereas pattern 2 predominated in PRCC-TFE3 tumors (19 of 24), similar to previous reports.9,11 All 82 tumors corresponded to International Society of Urological Pathology (ISUP) nuclear grade 2 or 3,12 and psammoma bodies were present in 49 tumors. Most tumors had no or only focal expression for epithelial markers (epithelial membrane antigen [EMA] and/or cytokeratins) and vimentin, with exceptions for each of these markers. It should be emphasized that several MiT-RCCs had regions that were morphologically similar to either CCRCC or PRCC.

TFEB MiT-RCC
Six tumors had TFEB gene rearrangements documented by FISH. Three of these cases exhibited a characteristic biphasic cell population, as previously described,13 consisting of large cells with eosinophilic and granular-to-clear cytoplasm and vesicular nuclei with prominent nucleoli (ISUP grade 2 or 3) admixed with less numerous small cells with little cytoplasm and dense chromatin (ISUP grade 1) (Fig. 1D). The remaining 3 tumors lacked a biphasic cell population and exhibited significant overlap with the features observed in TFE3 MiT-RCC, including 1 with predominant clear cell morphology. Five of 6 tumors exhibited focal or diffuse immunohistochemical expression for at least 1 marker of melanocytic differentiation (melan-A and/or human melanoma black 45 [HMB-45]).

Papillary RCC
Thirty-five tumors were classified as PRCC based on morphology and immunophenotype; 33 of these 35 tumors could be further classified as either type 1 or type 2 PRCC, as previously described.4 Type 1 PRCCs (19 tumors) had papillary structures lined by a single layer of cuboidal epithelium with scant cytoplasm (H&E stain, original magnification ×100); and (F) type 2 papillary RCC exhibiting pseudostratification of the papillae and eosinophilic cytoplasm (H&E stain, original magnification ×200).

Figure 1. Renal cell carcinomas (RCCs) with translocations involving members of the microphthalmia transcription factor MiT-RCC and papillary RCC (PRCC) are shown, including (A) nuclear transcription factor E3 (TFE3) immunohistochemical staining in a tumor positive for TFE3 rearrangement (original magnification ×200); (B,C) MiT-RCC exhibiting a combination of nested and papillary architecture, various amounts of clear and eosinophilic cytoplasm, and psammoma bodies, in which 2 main cytologic patterns are appreciated, including (B) 1 with voluminous cytoplasm and well defined cell membranes (hematoxylin and eosin [H&E] stain, original magnification ×200), and (C) 1 with smaller cells and less distinct cell membranes (H&E stain, original magnification ×100); (D) a biphasic cell population consisting of large and small cells observed in a translocated RCC with a transcription factor EB (TFEB) fusion transcript (H&E stain, original magnification ×200); (E) type 1 papillary RCC with papillary structures lined by a single layer of cuboidal epithelium with scant cytoplasm (H&E stain, original magnification ×100); and (F) type 2 papillary RCC exhibiting pseudostratification of the papillae and eosinophilic cytoplasm (H&E stain, original magnification ×200).
Renal Medullary Carcinoma

All 26 cases of RMC had the classic morphologic features previously described, including cells with eosinophilic cytoplasm and high nuclear grade arranged in a reticular growth pattern with prominent neutrophilic inflammation (hematoxylin and eosin [H&E] stain, original magnification × 200); (B) chromophobe RCC exhibiting cells with pale eosinophilic cytoplasm and perinuclear halos (H&E stain, original magnification × 200); (C) clear cell RCC with clear cells arranged in a solid growth pattern (original magnification × 100); (D) fumarate hydratase-deficient RCC with cells exhibiting frequent nucleoli and perinuclear clear halos lining papillary structures (original magnification × 400); (E) succinate dehydrogenase-deficient RCC exhibiting cells with eosinophilic cytoplasm and intracytoplasmic vacuoles that contain pale eosinophilic material (original magnification × 400); (F) tuberous sclerosis-associated RCC composed of cells with granular eosinophilic cytoplasm in a solid and nested growth pattern (original magnification × 200); (G) anaplastic lymphoma kinase (ALK)-rearranged RCC characterized by cells containing abundant eosinophilic cytoplasm, intracytoplasmic lumina, and vesicular chromatin (original magnification × 400); (G) thyroid-like RCC with follicular structures containing eosinophilic material, reminiscent of thyroid follicles (original magnification × 100); and (H) myoepithelial carcinoma exhibiting small epithelioid cells embedded in a myxoid stroma (original magnification × 200).

Figure 2. Other renal cell carcinoma (RCC) subtypes include (A) renal medullary carcinoma composed of cells with eosinophilic cytoplasm and high nuclear grade frequently arranged in a reticular growth pattern with prominent neutrophilic inflammation (hematoxylin and eosin [H&E] stain, original magnification × 200); (B) chromophobe RCC exhibiting cells with pale eosinophilic cytoplasm and perinuclear halos (H&E stain, original magnification × 200); (C) clear cell RCC with clear cells arranged in a solid growth pattern (original magnification × 100); (D) fumarate hydratase-deficient RCC with cells exhibiting frequent nucleoli and perinuclear clear halos lining papillary structures (original magnification × 400); (E) succinate dehydrogenase-deficient RCC exhibiting cells with eosinophilic cytoplasm and intracytoplasmic vacuoles that contain pale eosinophilic material (original magnification × 400); (F) tuberous sclerosis-associated RCC composed of cells with granular eosinophilic cytoplasm in a solid and nested growth pattern (original magnification × 200); (G) anaplastic lymphoma kinase (ALK)-rearranged RCC characterized by cells containing abundant eosinophilic cytoplasm, intracytoplasmic lumina, and vesicular chromatin (original magnification × 400); (G) thyroid-like RCC with follicular structures containing eosinophilic material, reminiscent of thyroid follicles (original magnification × 100); and (H) myoepithelial carcinoma exhibiting small epithelioid cells embedded in a myxoid stroma (original magnification × 200).
available in 3 patients, including 1 patient each with known von Hippel-Lindau disease (VHL) or multiple endocrine neoplasia type 1 syndrome (MEN1), and 1 whose tumor arose in a kidney allograft from an adult donor. One additional tumor had a translocation involving chromosome 3p13 (VHL gene locus) (Table 2). In all 4 patients, the tumors were small (ranging from 1.4 to 4 cm) and incidentally detected.

**Fumarate Hydratase-Deficient RCC**

Mutations in the fumarate hydratase (FH) gene were identified by NGS in 3 tumors (Table 2) that initially were classified as RCC-NOS, and the morphologic features were similar to those previously described. This includes papillary or tubulopapillary architecture admixed with lesser degrees of solid, nested, tubular, and/or cystic patterns. The majority of tumor cells contained eosinophilic cytoplasm, although focal areas with clear cytoplasm were observed. Prominent nucleoli with associated perinuclear clear halos were present in various amounts in all 3 tumors (Fig. 2D). In 1 tumor, frequent intracytoplasmic lumina and scattered vacuoles containing eosinophilic material reminiscent of succinate dehydrogenase-deficient RCC were observed. The tumor cells in all 3 tumors were positive for EMA, vimentin, and paired box 8 (Pax8) and negative for cytokeratin 7.

**Succinate Dehydrogenase-Deficient RCC**

A germline mutation in the succinate dehydrogenase (SDH) B (SDHB) gene was previously identified (Table 2) in 1 tumor that was initially classified as RCC-NOS, and the tumor exhibited the typical morphologic features previously described, characterized by a solid and trabecular architecture containing round-to-polygonal cells with moderate amounts of flocculent eosinophilic cytoplasm. Numerous intracytoplasmic vacuoles containing pale eosinophilic material were noted (Fig. 2E). Immunohistochemical stains revealed diffuse Pax8 expression and patchy cytokeratin 7 expression.

**Other Entities**

Twenty-two tumors initially classified as RCC-NOS were reclassified into recently recognized entities that are not included in the latest World Health Organization classification. Four tumors had ALK gene rearrangements, and 7 of 8 have been previously reported. Unifying pathologic features included the presence of abundant eosinophilic cytoplasm, intracytoplasmic lumina, vesicular chromatin, a lymphoplasmacytic infiltrate (Fig. 2G), diffuse moderate-to-strong TFE3 nuclear immunohistochemical expression, and retention of INI-1 nuclear protein expression. Immunohistochemistry for ALK was positive in 4 of 5 tumors tested. Fusion partners identified by NGS included vinculin (VCL) (3 tumors); tropomyosin 3 (TPM3) (3 tumors); hook homolog 1 (HOOK1) (1 tumor); and striatin, calmodulin-binding protein factor (STRN) (1 tumor) (Table 2).

**Thyroid-like RCC**

Three tumors were well circumscribed and composed of numerous tubular/follicular structures containing dense eosinophilic material reminiscent of thyroid follicles (Fig. 2H), as previously described. The cells had bland nuclear features with small-to-medium nucleoli. Two tumors diffusely expressed cytokeratin 7, and all 3 expressed EMA and vimentin.

**Myoepithelial carcinoma**

Two tumors classified as primary renal myoepithelial carcinomas have been previously reported. Both were
heterogeneous with epithelioid, clear cell, plasmacytoid, and spindle cell features embedded in a variably myxoid stroma (Fig. 2I). They expressed cytokeratins and S-100, and both had Kruppel-like factor 15–Ewing sarcoma breakpoint region 1 (KLF15-EWSR1) fusion transcripts identified by NGS (Table 2).

**Unclassified Tumors**

Among 42 tumors that were classified initially as RCC-NOS, 16 (38.1%) remain unclassified, corresponding to 7.5% of our entire cohort. All 16 tumors exhibited morphologic and/or immunophenotypic evidence of epithelial differentiation and expression of at least 1 marker of renal epithelial origin (RCC antigen and/or CD10). Further characterization was not possible in 3 of 16 tumors because of extensive necrosis and little viable tumor. Two tumors had the characteristic morphologic features of MiT-RCC; however, immunohistochemical expression of TFE3 or evidence of TFE3/TFEB gene rearrangements could not be documented. Two tumors exhibited marked morphologic overlap with the oncocytic TS-associated RCC described above, including 1 with bilateral, morphologically identical tumors. Both tumors were negative for TSC1/TSC2 mutations by NGS. Each of the remaining 9 of these tumors had distinctive and peculiar histopathologic features, lacked morphologic overlap, and did not allow for confident classification into any known entity. Six had sufficient material for NGS analysis, and no mutations were identified in the TSC1, TSC2, FH, folliculin (FLCN), SDHA, SDHB, SDHC, SDHD, met proto-oncogene (MET) (hepatocyte growth factor receptor), VHL, or phosphatase and tensin homolog (PTEN) genes.

**DISCUSSION**

In the current study, we used histology, immunohistochemistry, and focused genetic analysis to delineate the frequency of distinct RCC subtypes in a large prospective series of children and young adults enrolled in a multicenter collaborative protocol. Our results demonstrate that the most common subtype in this age group is MiT-RCC (41.5%), followed by PRCC (16.5%), RMC (12.3%), chromophobe RCC (6.6%), TS-associated RCC (4.2%), ALK-rearranged RCC (3.8%), and CCRCC (3.3%). The higher prevalence of MiT-RCC and the lower prevalence of PRCC and CCRCC in our series compared with smaller studies suggest that, before the current availability of specialized testing, some MiT-RCCs may have been classified as PRCC or CCRCC.5,6 CCRCC represents a minority of RCCs in this age group (3.3%), and these patients often have underlying predisposing syndromes or diseases. Finally, the relatively high frequency of RMC is in keeping with its increased prevalence in younger versus older patients.22

This study also contributes to our knowledge of the different translocations responsible for MiT-RCC. It is striking to note that only 6.8% of MiT-RCCs had TFE3 gene rearrangements. Among tumors that had confirmed TFE3 rearrangements, the ASPL and PRCC genes were the most common fusion partners, in keeping with the existing literature.9 Less frequent fusion partners include SFPQ, NONO, and MED15. This is consistent with the recently reported growing numbers of alternative partner genes, which expand the genetic spectrum of TFE3 MiT-RCCs.11,23 We were unable to identify the TFE3 fusion partner in 16 of 72 tumors (22%) that had a documented TFE3 rearrangement; however, none of those 16 tumors could be fully tested by both dual-probe FISH and NGS. The morphologic features described for TFE3 MiT-RCC are quite distinctive4,9 and were consistently present in our tumors. The combination of characteristic morphologic features, under expression of epithelial markers, and diffuse and strong nuclear TFE3 immunohistochemical expression reliably detected RCC with TFE3 rearrangements in our series without the need for molecular studies. False-negative FISH results using TFE3 break-apart probes have been reported in MiT-RCCs bearing specific fusion transcripts (ie, NONO-TFE3 and RBM10-TFE3)24-26 and were attributed to a short distance between the sequences targeted in rearrangements involving chromosome inversions, with a resulting lack of split signals. These observations may explain why 2 of 82 tumors classified as TFE3 MiT-RCC (2.4%) exhibited TFE3 positivity and characteristic histologic and immunophenotypic features yet were negative for a TFE3 rearrangement by FISH. Although it has been reported that TFE3 immunohistochemical overexpression is highly sensitive and specific (97.5% and 99.6%, respectively) for the diagnosis of TFE3 MiT-RCC,27 this should be carefully considered in context with the appropriate histologic and immunophenotypic appearance. Diffuse TFE3 positivity was observed in our ALK-rearranged RCCs, which have morphologic and immunophenotypical features entirely distinct from those described in TFE3 MiT-RCC.

Approximately 4% of all RCCs are believed to have a hereditary basis,28 and most of these tumors typically present in early adulthood. Therefore, a higher incidence of hereditary RCC would be expected in patients younger than 30 years. Only 15 of 212 patients (7.1%) in our study had either a clinical diagnosis or a molecular
abnormality within tumor DNA suggestive of a cancer predisposition syndrome. These include TS (9 patients); FH deficiency (3 patients); and SDHB deficiency, VHL disease, and MEN1 (1 patient each). It remains quite possible that additional patients with other cancer predisposition syndromes exist in this cohort and, conversely, that patients with a known family history of cancer predisposition syndrome may not register for COG protocols. TS is of particular interest considering its highly variable penetrance, the need to distinguish RCC from epithelioid angiomylipomas, and the heterogeneity of previously reported TS-associated RCCs. Of the 212 patients in the current study, 9 had either a history of TS or a documented TSC2 mutation. Two additional patients demonstrated morphologically similar tumors (1 of which was bilateral), suggesting the possibility of mutations in related genes or undetected mutations in TSC1/TSC2.

The current study provides the first diagnostic classification of RCCs in patients aged < 30 years who were prospectively registered on a cooperative group clinical study. The results expand the pathologic spectrum of RCC in this group by recognizing additional entities, most of which required genetic analysis for confident classification. The recognition of these entities resulted in a lower percentage (7.5%) of unclassified RCCs in our series compared with 16% to 24% reported in smaller series, supporting the need for a thorough diagnostic workup of tumors that have unusual morphology. In summary, using a focused genetic approach, it is feasible to efficiently identify specific molecular markers in the majority of tumors from the pediatric and young adult patients studied. Some of the genetic abnormalities identified in these tumors may allow for targeted therapy in the future.

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CONFLICT OF INTEREST DISCLOSURES
The authors made no disclosures.

AUTHOR CONTRIBUTIONS
Mariana M. Cajaiba: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, and writing—review and editing. Lisa M. Dyer: Investigation, methodology, and writing—original draft. James I. Geller: Methodology, resources, supervision, and writing—review and editing. Lawrence J. Jennings: Investigation, methodology, and writing—original draft. David George: Investigation. Dawn Kirschmann: Investigation. Stephen M. Rohan: Investigation. Nicholas G. Cost: Methodology. Geetika Khanna: Methodology. Elizabeth A. Mullen: Methodology, and writing—review and editing. Jeffrey S. Dome: Methodology and writing—review and editing. Conrad V. Fernandez: Methodology, supervision, and writing—review and editing. Elizabeth J. Perlman: Conceptualization, investigation, methodology, resources, supervision, writing—original draft, and writing—review and editing.

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