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Pediatric cystic nephroma are morphologically, immunohistochemically, and genetically distinct from adult cystic nephroma

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Abstract

The term cystic nephroma has traditionally been used to refer to two neoplasms, a lesion in adults that is now thought to be part of the spectrum of mixed epithelial stromal tumor (MEST) and a pediatric lesion which has been associated with mutations in the *DICER1* gene. A direct detailed morphologic, immunohistochemical, and genetic comparison of these two lesions has not been performed. In this study, we compare the morphologic features, immunoreactivity for estrogen receptor and inhibin, and *DICER1* genetic status of 12 adult cystic nephroma/MEST (median age 50.5 years, all females) and 7 pediatric cystic nephroma (median age 1.3 years, male: female=6:1). Both lesions (11 of 12 adult cases, 6 of 7 pediatric cases) frequently demonstrated subepithelial accentuation of stromal cellularity, though the increased cellularity frequently included inflammatory cells in the pediatric cases. All adult and pediatric cases labeled for estrogen receptor; however, while most (83%) of adult cases labeled for inhibin at least focally, no pediatric case labeled for inhibin. Most adult cases (58%) demonstrated wavy, ropy collagen in association with cellular stroma, whereas this was not found in pediatric cases. 86% of pediatric cases demonstrated *DICER1* mutations, whereas only 1 of 10 adult cases demonstrated a *DICER1* mutation. In summary, while cellular stroma and estrogen receptor immunoreactivity are commonly present in both adult and pediatric cystic nephroma, ropy collagen and inhibin immunoreactivity are far more common in adult cystic nephroma/MEST, whereas *DICER1* mutations are far more prevalent in pediatric cystic nephroma. These results support the current World Health Organization Classification's separation of adult and pediatric cystic nephromas as distinct entities.

Keywords

Renal Neoplasm; Cystic Nephroma; *DICER1*

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Introduction

In the field of renal neoplasia, the term cystic nephroma has historically been problematic¹. As first deduced and asserted by Dr. John Eble in 1994², the term cystic nephroma has been used to refer to two apparently distinct lesions³. The first, adult cystic nephroma, typically affects adult females (suggesting an association with circulating hormones), and has been thought by many to be the highly cystic end of the spectrum of mixed epithelial stromal tumor (MEST)⁴⁻¹³. In contrast, pediatric cystic nephroma typically affects very young children (usually less than 24 months of age), and has traditionally been thought to be part of the spectrum of cystic nephroblastic lesions which includes cystic partially differentiated nephroblastoma (when immature nephroblastic elements are present in the septa of a purely cystic neoplasm), and cystic Wilms tumor (when nephroblastic elements form nodules in the septa that indent the cysts)^{14,15}. In the 2004 World Health Organization (WHO) classification of renal neoplasia, pediatric cystic nephroma is not recognized as a distinctive entity; instead, it is considered to be part of the spectrum of cystic partially differentiated nephroblastoma and cystic Wilms tumor¹⁶. Adult cystic nephroma is considered a separate entity classified under soft tissue tumors of the kidney¹⁷. In the 2016 WHO classification of renal neoplasia, adult cystic nephroma is considered a part of the mixed epithelial and stromal tumor (MEST) family on the basis of similar age and sex distributions, overlapping morphology, and similar immunohistochemical profiles¹⁸. Pediatric cystic nephroma is now considered a distinctive entity, associated with mutations in the *DICER1* gene¹⁹. Germline *DICER1* mutations have been identified in young patients with pleuropulmonary blastoma and its other associated neoplasms, including pediatric cystic nephroma; this constellation of lesions is now termed *DICER1* syndrome²⁰. Interestingly, *DICER1* mutations have not been found in cystic partially differentiated nephroblastoma, suggesting that these two entities, previously thought to be part of morphologic spectrum of a single disease, are in fact distinctive²¹.

Few studies have performed a detailed comparison of pediatric and adult cystic nephroma at the morphologic, immunohistochemical and genetic levels. One study reported the absence of *DICER1* mutations in 10 adult cystic nephroma, but provided no illustrations or immunohistochemical analysis of these cases²². Another study found no evidence of *DICER1* mutations in 29 adult cystic nephroma/MEST in adults, but provided no immunohistochemical analysis and did not compare these to pediatric cystic nephroma²³. The absence of detailed morphologic and immunohistochemical analysis in these studies is relevant given the variety of renal lesions that can closely mimic the morphology of both pediatric and adult cystic nephroma²⁴.

In this study, we perform a detailed morphologic and immunohistochemical analysis of adult cystic nephroma/MEST family lesions and pediatric cystic nephroma. We also evaluate *DICER1* genetic status in both sets of neoplasms.

Materials and Methods

Institutional review board approval

This study was approved by the Institutional Review Board of the Johns Hopkins Hospital and other participating institutions.

Cases

We reviewed 18 cases originally classified as adult cystic nephroma/MEST and 8 cases originally classified as pediatric cystic nephroma from 2 institutions (The Johns Hopkins Hospital, Children's Hospital of Philadelphia). Adult cystic nephroma/MEST was defined as a well circumscribed, multilocular tumor with noncommunicating cysts lined by flat and hobnailed epithelium. The septa were typically fibrous and focally cellular with wavy ovarian-like stroma, or showed smooth muscle differentiation¹⁸. Pediatric cystic nephroma was defined as a well delineated, multilocular exclusively cystic neoplasm of young children in which the septa were composed of fibrous tissue and differentiated tubules¹⁹. Six cases originally classified as adult cystic nephroma/MEST and one case originally classified as pediatric cystic nephroma were subsequently reclassified into other diagnostic categories (see *Results*), leaving 12 adult cystic nephroma/MEST cases and 7 pediatric cystic nephroma cases for study. On all cases, immunohistochemistry for estrogen receptor (Ventana, clone 6F11, 1:50 dilution) and inhibin (Serotec, clone R1, 1:20 dilution) was performed, and mutation status of *DICER1* assessed when possible.

DICER1 mutation analysis

For each case, four 10-micron scrolls of formalin-fixed, paraffin embedded samples, were prepared from a representative tumor block. Following paraffin removal, DNA was extracted using the Maxwell 16 Formalin-Fixed Paraffin Embedded Tissue LEV DNA Purification Kit (Promega, Madison, WI) on the Maxwell 16 Instrument (Promega). DNA quality and quantity was assessed using a Nanodrop (ThermoFisher, Wilmington, DE) and Qubit (Qubit 2.0, Life Technologies) respectively. DNA libraries were prepared using a custom multiplex polymerase chain reaction panel designed for the coding regions of *DICER1* with an average amplicon length less than 200 base pairs (Ion Torrent Ampliseq, Life Technologies, Grand Island, NY, USA).²⁵ NGS was performed on an Ion Torrent 318 v2 chip (ION PGM Sequencing 200 kit v2, Life Technologies) with an average of 6 samples per chip, to achieve an average depth of coverage of 3000 filtered reads. Signal processing, mapping and quality control were performed with Torrent Suite software v.4.0.2 (Life Technologies). Variant calls were made using the Torrent Variant Caller Plugin v.4.0, with somatic low stringency mutation workflow and default settings. BAM files of raw reads were reviewed using Integrative Genomics Viewer v2.3.^{26,27}. Variants were annotated with Alamut Software and named using HUGO nomenclature for *DICER1* transcript NM_177438.2. Nonsense and frameshift mutations were classified as “allele loss of function.” Missense variants affecting codons for amino acids 1705, 1709, and 1809, 1810 and 1813 were classified as “hotspot mutations.” Allele frequency was recorded for each variant. Coverage information was recorded for each of the cases with normal sequence at all hotspot bases. SIFT was used to assess the potential significance of predicted novel amino acid substitutions outside of the hotspot regions <http://sift-dna.org>^{28,29,30}.

Results

Adult cystic nephroma/MEST

The clinicopathologic features of these cases are illustrated in Table 1. All 12 confirmed adult cystic nephroma/MEST were well-delineated, biphasic benign neoplasms that occurred in females, with a mean age of 52 years and a median age of 50.5 years. Almost all adult cystic nephroma/MEST showed accentuation of stromal cellularity in the subepithelial zone (11 of 12 cases, 92%). Most cases (7 of 12, 58%) demonstrated wavy, ropy collagen in association with cellular stroma, similar to that found in adult ovarian stroma. In all cases, the stroma labeled for estrogen receptor, typically in a weak-moderate but diffuse fashion. The majority of cases (10 of 12, 83%) demonstrated at least focal labeling for inhibin, typically in more plump cells which resembled luteinized stromal cells (Figures 1-2). Nine of 10 evaluable cases did not show evidence of *DICER1* mutation. In the one case (case 10) that did demonstrate evidence of *DICER1* mutation, RNA was of low quality, but identical results were obtained from two separate samples cut from the sample tissue block at different times and using nucleic acid precautions (Figure 3). This case was somewhat unusual in that it did not demonstrate ropy collagen and was non-immunoreactive for inhibin, but otherwise was typical of adult cystic nephroma/MEST as it demonstrated estrogen receptor-positive smooth muscle stroma, cysts and complex epithelial patterns (Figure 3). Two cases could not be analyzed for *DICER1* mutation as nucleic acids were excessively degraded.

Pediatric Cystic Nephroma

The clinicopathologic features of these cases are illustrated in Table 1. The median patient age was 1.4 years (mean 1.4 years), and 6 of 7 cases were males. One case was bilateral. On microscopic examination, all of these cases were exclusively cystic, demonstrating bland fibroblastic stroma (Figures 4-5). In one case, a primitive glomerulus was present near the edge of the lesion, but otherwise no primitive nephroblastic elements were identified in the septa. Six of 7 cases demonstrated focal increases in subepithelial cellularity, some due to spindle cells and some due to admixed chronic inflammation. The stroma of all pediatric cystic nephroma labeled for estrogen receptor, typically more diffusely and intensely than that seen in the adult cystic nephroma/MEST cases. However, none of the pediatric cystic nephroma cases demonstrated labeling for inhibin. Six of 7 pediatric cystic nephromas demonstrated *DICER1* mutation (86%). The case that was negative demonstrated no distinguishing clinical or morphologic features, though it was the least well-sampled tumor in the study (4 sections from a 12 cm tumor). The case with the rare entrapped primitive glomerulus did demonstrate a *DICER1* mutation.

Cases Excluded from the Study

Six cases originally classified as adult cystic nephroma/MEST were excluded from the study. Among the adult cases, 3 cases demonstrated nodules of tubulopapillary epithelium, absence of ovarian-like stroma, and absence of estrogen receptor labeling by immunohistochemistry and hence were reclassified as smooth muscle and adenoma like renal tumors (SMART)³¹. Two cases contained fibrous septa and demonstrated a bland clear cell lining that was diffusely immunoreactive for carbonic anhydrase IX, and were

reclassified as variants of multilocular cystic renal neoplasm of low malignant potential³². One case demonstrated subtly infiltrative borders, fibroelastotic stroma, and polygonal neoplastic cells with eosinophilic cytoplasm, prominent nucleoli and irregular nuclear contours, and was reclassified as a tubulocystic carcinoma. Among the pediatric cases, one lesion originally classified as cystic nephroma was noted to permeate among native nephrons and lack a well-defined fibrous wall, and hence was reclassified as segmental polycystic kidney disease³³ (Figure 6). Of note, all of the above cases were tested and none demonstrated evidence of *DICER1* mutation.

Discussion

The differential diagnosis of cystic nephroma is broad, and includes cystic partial differentiated nephroblastoma (in children), segmental polycystic kidney disease, multilocular cystic renal neoplasm of low malignant potential, SMART (for more solid lesions in adults), and tubulocystic carcinoma. Whether adult cystic nephroma should be grouped together with MEST remains a controversy. Some authors believe that these are distinctive entities, based upon the ability to separate lesions into one of these two categories in most cases (MEST being more solid and complex than cystic nephroma), differences in morphology and immunoprofile (frequent smooth muscle differentiation in MEST and inhibin labeling in cystic nephroma)^{34,35}, and potential differences in etiology (some postulate that MEST may not have appeared until after 1950, and may be linked to exposure to exogenous hormones like oral contraceptives) (John N. Eble, personal communication)³⁶. However the current 2016 WHO Classification states “on the basis of similar age and sex distributions, as well as similar immunohistochemical profile and overlapping histologic features, adult cystic nephroma is now classified within the spectrum of MEST family”. Therefore, we have followed the 2016 WHO classification and grouped our adult cystic nephroma and MEST cases together and compared them to pediatric cystic nephroma. We recognize of course that this area remains controversial, and note that, among our adult cases, cases 10 and 11 would likely be classified as MEST and cases 1-9 and 12 would likely be classified as cystic nephroma if this distinction were made.

In this study, we perform a direct comparison of pediatric and adult cystic nephroma/MEST. We find several similarities which have not been well-described in the literature. First, we note that pediatric cystic nephroma typically show areas of subepithelial cellularity which simulates the cellular, wavy, ovarian-like stroma seen in adult cystic nephroma/MEST. We note that a recent review of pediatric cystic nephromas indicates in the text that cellular foci were not identified, though one of the figures illustrates it and the figure legend describes “frequent pericystic stromal condensation”²². Second, we found that both pediatric and adult cystic nephroma/MEST demonstrate diffuse stromal immunoreactivity for estrogen receptor. The latter is well known in adult cystic nephroma/MEST, but to our knowledge has not been described in pediatric cystic nephroma. These similarities may help explain why adult and pediatric cystic nephromas were originally classified together under the same designation, as the stroma has a cellular appearance in both which can somewhat resemble ovarian stroma. However, we note several morphologic and immunohistochemical differences between these lesions. First, the stroma of pediatric cystic nephroma lacks the wavy, ropy collagen which is typical of adult cystic nephroma/MEST, and some of the

increased cellularity consists of inflammatory cells, possibly due to incipient rupture of cysts. Second, labeling for inhibin was in our series absent in pediatric cystic nephroma while it is typically focally present in adult cystic nephroma/MEST. We suspect that the diffuse ER labeling in pediatric cystic nephroma (and to some extent adult cystic nephroma/MEST) represents the effects of obstruction, which has been known to induce estrogen receptor labeling in cystic renal lesions.³⁷ In summary, the stroma of adult cystic nephroma/MEST has the phenotype of ovarian stroma, while that of pediatric cystic nephroma does not but morphologically and immunohistochemically can simulate it.

Our results generally agree with those previously published in the literature. Doros *et al.* reported *DICER1* mutations in 18 of 20 (90%) pediatric cystic nephromas, but did not study adult lesions.²¹ Vanecek *et al.* found no evidence of *DICER1* mutation in 29 adult cystic nephromas/MESTs²³. However, this study included no pediatric cystic nephromas and no immunohistochemical analysis. Another study found no mutations in *DICER1* in 10 “adult cystic nephroma”²². However, no clinical information besides age and gender were provided for these cases, and immunohistochemistry was not performed. Given the mimics of cystic nephroma described in the current paper and in the literature,²⁴ the absence of thorough immunohistochemical and morphologic analysis of the cases reported in these studies is problematic.

We found two outlier cases in our study. The first outlier case was a pediatric cystic nephroma that lacked a *DICER1* mutation (pediatric case 1). This lesion was no different in any clinical or morphologic sense from the other pediatric cystic nephromas in our study. However, it is possible this case could have been a cystic partially differentiated nephroblastoma (which have previously been shown to be *DICER1* negative²¹), in which small areas of nephroblastomatous elements were not sampled¹⁴. Of note, this was the least well-sampled tumor in our study: only 4 sections were taken from this 12 cm cystic neoplasm. It should also be noted that 90% of pediatric cystic nephroma reported in the study of Doros *et al.*²¹ harbored *DICER1* mutations, so 10% were negative. If these cases were not in fact inadequately-sampled cystic partially differentiated nephroblastomas, another hypothesis is that these *DICER1*-negative pediatric cystic nephromas harbor other genetic alterations which effect the same pathway and create the same phenotype. The second outlier case was an adult cystic nephroma/MEST (adult case 10) which demonstrated a *DICER1* mutation. This case was different from most such cases in that it did not label for inhibin and have ropy collagen as the majority of such lesions in our study did, but demonstrated estrogen receptor-positive smooth muscle stroma and complex cystic epithelial patterns, and would likely have been put in the MEST category by those who subdivide adult cystic nephroma and MEST. It should be noted that one similar case has been reported in the literature in abstract form³⁸. This case was a 20 year old women who developed a sarcoma following diagnosis of a MEST that harbored a *DICER1* mutation. Hence, while it is true that the vast majority of adult cystic nephroma/MEST lack *DICER1* mutations, rare cases currently classified as adult MEST may harbor *DICER1* alterations, which likely are sporadic. One can postulate that such lesions originated as cystic nephromas in childhood, but remained undetected until adult life and perhaps underwent morphologic changes (such as smooth muscle metaplasia) in the interval. Hence, *DICER1* mutation status does not absolutely distinguish adult and pediatric cystic nephroma in all cases, though in the vast

majority of cases it does. The marked differences in age, gender predominance, morphology and immunohistochemical profile justify the current distinction of pediatric cystic nephroma from adult cystic nephroma/MEST. Further analysis of additional outlier cases (i.e., adult cystic nephroma/MEST harboring *DICER1* mutations) would be required to determine if *DICER1* status is instead the best way to divide these cases.

In summary, we report distinguishing morphologic, immunohistochemical and genetic features between adult cystic nephroma/MEST and pediatric cystic nephroma. We find that ropy collagen and inhibin immunoreactivity are far more common in adult cystic nephroma/MEST, whereas *DICER1* mutations are far more prevalent in pediatric cases. Cellular stroma and estrogen receptor immunoreactivity are present in both entities, and may reflect in part the effects of cystic dilatation and obstruction.

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Figure 1.

Adult cystic nephroma/MEST lacking *DICER1* mutation (41 year old female). This is a cystic renal neoplasm which has septa which are thicker than those of pediatric cystic nephroma, and composed of cellular, ovarian-like stroma associated with ropy collagen (A, B). The septa stromal cells are immunoreactive for estrogen receptor (C) and inhibin (D).

Figure 2.

Adult cystic nephroma/MEST lacking *DICER1* mutation (73 year old female). This is an exclusively cystic neoplasm (A) composed of thin fibrous septa with cellular stroma morphologically resembling ovarian stroma (B). The septa are immunoreactive for estrogen receptor (C), and show focal labeling in more epithelioid cells for inhibin (D).

Figure 3.

Adult cystic nephroma/MEST with *DICER1* mutation (50 year old female). The lesion is both solid and cystic, with the solid areas containing predominantly smooth muscle and the epithelial elements forming variably sized cysts (A-C). On high power view, one can appreciate cellular smooth muscle stroma, but the ropy collagen seen in most adult cystic nephroma/MEST is absent (D, E). The stroma is diffusely immunoreactive for estrogen receptor (F), but was negative for inhibin (not shown).

Figure 4. *DICER1*-mutated pediatric cystic nephroma (1 year old female). This is an exclusively cystic neoplasm (A) in which the septa are composed predominantly of fibrous tissue with focal subepithelial increased cellularity (B). Ropy collagen is absent. The subepithelial stroma labels for estrogen receptor (C), but not for inhibin (D).

Figure 5. *DICER1*-mutated pediatric cystic nephroma (2 year old male). This is a well delineated cystic neoplasm in which the cysts mold the septa (A, B). The septa are predominantly comprised of fibrous tissue with areas of increased cellularity (C). Ropy collagen is absent, and some of the increased cellularity is chronic inflammation (D, E). The septa label for estrogen receptor (F) but not for inhibin (not shown).

Figure 6.

Segmental polycystic kidney disease mimicking pediatric cystic nephroma (5 year old male, bilateral lesions). This is a multicystic lesion which the cysts intermingle with the native nephrons (A, B). One can appreciate native renal tissue within the septa of this lesion (C, D) which excludes the diagnosis of a neoplasm like cystic nephroma which typically pushes aside native nephrons. This lesion lacked a *DICER1* mutation.

Table 1

Cases in this study

Diagnosis	Age/Sex	Tumor Diameter (cm)/Number of Sections Taken	Ropy Collagen	Estrogen Receptor IHC	INHIBIN IHC	DICER1 RNase IIIb mutation (% allele frequency)	DICER1 LOF mutation (% allele frequency)
MEST/CN 1	41/F	6.5/8	yes	80% strong	Focal moderate	Negative	Negative
MEST/CN 2	61/F	8/5	yes	60% moderate	Focal moderate	Negative	Negative
MEST/CN 3	67/F	14/16	yes	60% moderate	Focal strong	Failure	Failure
MEST/CN 4	53/F	7/5	no	80% moderate-strong	Focal moderate	Low quality but negative	Low quality but negative
MEST/CN 5	45/F	1.5/4	yes	70% strong	Focal moderate	Failure	Failure
MEST/CN 6	41/F	7/10	yes	80% strong	Diffuse strong	Low quality but negative	Low quality but negative
MEST/CN 7	47/F	4/8	yes	50% moderate	Focal weak	Negative	Negative
MEST/CN 8	51/F	8/10	no	30% weak-moderate	Focal strong	Low quality but negative	Low quality but negative
MEST/CN 9	73/F	5/5	no	20% moderate	Focal strong	Negative	Negative
MEST/CN 10	50/F	3.3/5	no	100% strong	Negative	c.5437G>A; p.E1813K 6%	c.3143G>A; p.W1048*; 3.2%
MEST/CN 11	39/F	20/20	no	20% moderate	Negative	Negative	Negative
MEST/CN 12	58/F	2.6/10	yes	30% moderate	Focal weak	Negative	Negative
Pediatric CN 1	1.25/M	12/4	no	50% strong	Negative	Low quality but negative	Low quality
Pediatric CN 2	0.5/M	12/12	no	20% weak-moderate	Negative	c.5125G>A; p.D1709N; 62.2%	Low quality but negative
Pediatric CN 3	2/M	6/13	no	20% moderate	Negative	c.5437G>A; p.E1813K; 13.8%	c.3452_3453delTG; p.V1151Efs12*; 50.5%
Pediatric CN4 (bilateral)	1.2/M	12/8	no	60% strong	Negative	c.5425G>A; G1809R; 40%	Negative
Pediatric CN 5	1.3/M	10/24	no	10% moderate	Negative	c. 5125G>A; p.D1709N 26.9%	c.958A>T; p.K320* 45.8%
Pediatric CN 6	2.2/M	10/14	no	50% moderate	Negative	c.5428G>C; p.D1810H; 4.6%	c. 3091C>T; p.Q1031*; 6.1%
Pediatric CN 7	1.3/F	2/2	no	70% strong	Negative	c.5125G>A; p.D1709N; 10.3%	c.1177C>T; p.Q393*; 50.7%

Age is in years; M=male; F=female; IHC=immunohistochemistry