Improvement in Renal Cystic Disease of Tuberous Sclerosis Complex After Treatment with Mammalian Target of Rapamycin Inhibitor

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Renal cysts occur in approximately 50% of patients with tuberous sclerosis complex, but their clinical significance and response to treatment are unknown. Abdominal imaging of 15 patients with tuberous sclerosis complex-associated renal cystic disease who had received mammalian target of rapamycin inhibitor therapy for other tuberous sclerosis complex-related indications was evaluated. Reductions in cyst number, sum diameter, and volume were observed. *(J Pediatr 2017;■■:■■-■■).*

Tuberous sclerosis complex (TSC) is a multisystem, autosomal dominant disorder associated with mutations in either the *TSC1* or *TSC2* genes. Loss of function of these genes, which code for hamartin and tuberin, respectively, results in inappropriate activation of the mammalian target of rapamycin (mTOR) complex, which can lead to uncontrolled cell growth and division, as well as excessive protein synthesis in affected organs systems.[1,2] Angiomyolipomas are the most common renal manifestation of TSC, occurring in approximately 80% of patients.[3-7] It is postulated that angiomyolipoma growth may impair renal function by replacing normal parenchyma.[3] They are associated with a risk of hemorrhage that is positively correlated with increasing lesion size.[7,8]

The second most common renal manifestation of TSC is parenchymal cysts, occurring in approximately 50% of patients.[3,6,9] Most occur owing to gene mutation of *TSC1* or *TSC2* alone,[9] but in about 2% of patients with TSC they arise with the contiguous gene syndrome (CGS), in which the genetic mutation spans both *TSC2* and *PKD1* on chromosome 16.[10] Patients with CGS exhibit a more severe renal phenotype that leads to very early onset, severe polycystic kidney disease and the development of end-stage renal failure in young adulthood, resembling the cystic disease of autosomal dominant polycystic kidney disease (ADPKD). Renal cysts in TSC that are not associated with CGS (*PKD1*) can range from few in number and small in size to multiple bilateral cysts of varying size resembling ADPKD.[9,11] They can be macroscopic or microscopic and increase both in size and in number with age.[6,9,12] There are reports that cysts are associated more often with hypertension and chronic kidney disease than angiomyolipomas alone,[13] suggesting that they are an under-recognized and more severe manifestation of TSC than previously appreciated.

Small molecule inhibitors of mTOR, such as sirolimus and everolimus, have proven to be effective treatment for multiple manifestations of TSC.[14-18] Treatment with an mTOR inhibitor for renal manifestations of TSC has largely focused on the reduction of angiomyolipoma size and preventing spontaneous bleeding associated with large tumors that can lead to additional life-threatening medical complications.[19,20] The adoption of mTOR inhibitors as an alternative to surgery (full or partial nephrectomy) or selective renal arterial embolization has led to international recommendation of their use as first-line therapy for renal angiomyolipoma >3 cm in diameter.[21] The effectiveness of mTOR inhibitors as a treatment for renal cystic disease in TSC in unknown, and multiple clinical trials in adults with ADPKD to date have failed to demonstrate clinical benefit.[22-24]

We describe a population of patients with TSC with renal cystic disease not associated with CGS that received an mTOR inhibitor for indications other than renal cysts, yet all the patients demonstrated improvements in cyst burden.

ADPKD	Autosomal dominant polycystic kidney disease
CGS	Contiguous gene syndrome
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
TSC	Tuberous sclerosis complex

Methods

All data evaluated in this study were collected for clinical care purposes before institutional review board approval of this study. A cohort of patients with known renal cystic disease and TSC were identified via an institutional database spanning all clinical patient encounters between 2010 (first US Food and Drug Administration approval of everolimus in TSC) and 2016 (data cutoff). Only patients with baseline magnetic resonance

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imaging (MRI) and at least 1 additional MRI completed after the initiation of treatment with mTOR inhibitor therapy were included. If multiple post-therapy abdominal MRIs were obtained, the most recent study was used for comparison with the baseline. Charts and abdominal MRI images were reviewed to confirm that each patient met the genetic or clinical diagnostic criteria for TSC,[21] renal cysts, and received treatment with an mTOR inhibitor (sirolimus and/or everolimus). Additional clinical data collected for each patient included demographic information, date of cystic renal disease diagnosis, date of initiation and total duration of mTOR therapy, and any additional TSC-associated or other medical comorbidities. If genetic analysis had already been performed, the gene mutation was also recorded.

Abdominal MRI examinations were performed according a standard TSC imaging protocol of our institution and includes the following sequences: coronal T2-weighted, coronal T1-weighted, axial T2-weighted with and without fat saturation, axial in and opposed phase, axial and coronal steady-state free procession, and axial diffusion weighted imaging. All MRIs were reviewed independently by 2 board-certified pediatric radiologists. Two different methods were used to quantify renal cyst burden on MRIs at each timepoint with each of the reviewers blinded to the quantification performed by the other reviewer (**Figure 1**; available at www.jpeds.com). Using axial T2-weighted images, the first method involved manual characterization of renal disease burden, including quantification of the number of individual angiomyolipoma(s) and cyst(s) in each kidney. The kidney diameter was measured in 3 dimensions. Then, the maximum transverse diameter of the 10 largest renal cysts was measured and the maximum sum diameter of renal cysts for each patient at each time point was determined by adding the maximum diameter measurements of the 10 largest cysts from both the right and left kidneys. When measuring renal cyst diameter, there was no minimum size requirement for measurement. The second method involved semiautomated determination of total cyst volume of each kidney. Image evaluation software (ImageJ, National Institutes of Health, Bethesda, Maryland)[25] was used to perform a threshold analysis on the axial T2-weighted images to allow selection of only fluid attenuation pixels. Threshold variables varied by examination owing to differences in image acquisition variables and were optimized on a per-examination basis to select fluid attenuation pixels. Cyst volume was measured by manual placement of regions of interest over each kidney to measure the area (in square millimeters) occupied by fluid intensity pixels per image and then multiplying that area by slice thickness. If the renal pelvis was dilated, the central portion of the kidney was excluded manually so that only the bright pixels corresponding with renal cysts were included.

Statistical Analyses

Changes in maximum sum diameter, total cyst volume, and total number of cysts were evaluated using a Wilcoxon matched-pairs signed rank test (2-tailed) with Prism 7 statistical analysis software (GraphPad Inc, San Diego, California).

Results

Fifteen patients with TSC met the inclusion criteria, the clinical characteristics of which are summarized in **Table I** (available at www.jpeds.com). Of the 15 patients, 10 were female, and 13 were white. The remaining 2 were Asian and Hispanic. Although all the patients had confirmed renal cysts, 10 had accompanying angiomyolipomas. Some patients had renal ultrasound examinations before the first MRI in which renal cysts were already identified, resulting in mean age at the time of renal cyst diagnosis of 5.5 years (range, 1.5-16.8) and mean age at the time of first MRI of 7.7 years (range, 1.5-19.8). The mean age at commencement of mTOR inhibition was 7.8 years (range, 1.1-20.3). Subependymal giant cell astrocytomas were the primary indication for treatment in 11 of 15 patients (73.3%). All patients were treated with either everolimus or sirolimus for mTOR inhibition and treatment was ongoing in all of those included at time of data cutoff.

At the time of the initial MRI, 14 of the 15 patients had multiple, bilateral renal cysts ranging in number (sum of right and left renal cysts) from 2 to 454 with an average of 72 cysts per patient (**Table II**). The 15th patient had a single cyst in each of the right and left kidneys. The mean time on treatment between baseline and follow-up MRI examinations was 34.9 ± 14.7 months (median 29; range, 17-63).

Table II. Changes in cyst volume, sum diameter, and total number of cysts between the initial and follow-up MRI scans

	Mean (SD)	Median	Range	IQR
Volume 1 (mL)*	5.27 (10.82)	0.47	0.008 to 37.66	0.14 to 1.77
Volume 2 (mL)*	1.23 (3.92)†	.00	0.00 to 15.35	0.00 to 0.15
Δ Volume (mL)*	–4.04 (7.85)	–0.47	–0.008 to –23.90	–0.12 to –2.27
% Reduction*	92.0% (13.8%)	100%	59.2% to 100%	92.3% to 100%
Sum diameter 1 (mm)	78.0 (82.2)	46.2	3.6 to 324.3	30.3 to 90.1
Sum diameter 2 (mm)	35.8 (57.7)‡	11.0	0.0 to 201.9	1.0 to 36.7
Δ Sum diameter (mm)	–42.1 (38.6)	–26.8	–3.6 to –141.5	–25.2 to –37.5
% Reduction	69.9% (29.4%)	70.7%	20.3% to 100%	47.1% to 97.8%
Total no. of cysts 1	72.1 (109.8)	26	2 to 454	17.0 to 86.5
Total no. of cysts 2	10.9 (12.0)§	5	0 to 41	0.5 to 16.5
Cyst no. reduction	61.2 (114.2)	19	1 to 453	11.5 to 69.5

*Data from 1 patient were excluded from this analysis.
†$P = .0001$ compared with volume 1.
‡$P < .0001$ compared with sum diameter 1.
§$P = .0003$ compared with total no. of cysts 1.

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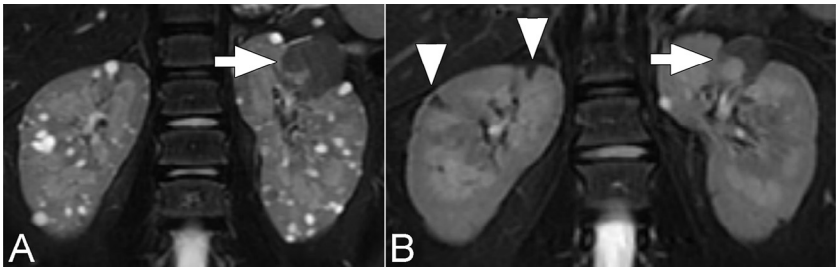

Figure 5. Coronal T2-weighted MRIs of the kidneys before **A,** and after **B,** mTOR inhibition show a marked decrease in the number of cysts in each kidney. A dominant angiomyolipoma in the upper pole of the left kidney also decreased in size (*arrows* in **A** and **B**). Smaller angiomyolipomas in the right kidney (*arrow heads* in **B**) were also decreased in size, but are not included in the selected pre-mTOR inhibitor image (**A**).

A reduction in the sum of cyst diameters occurred in all 15 patients (**Figure 2**; available at www.jpeds.com). The average reduction in the sum cyst diameter was 42.1 mm (median 26.8; range, 3.6-141.5), a 69.9% decrease. Similarly, total cyst volume decreased in 14 of 15 patients (**Figure 3**; available at www.jpeds.com). Among the 14 patients, cyst volume was reduced by an average of 4.0 mL (median, 0.47 mL; range, 0.008-23.900 mL), or 92%. In the 15th patient, 1 cyst increased in size (4.3 mm at baseline to 60.6 mm at follow-up), yet in the same patient all other cysts demonstrated a volumetric reduction. Overall, the number of cysts was reduced by 61 cysts per patient on average, a 72.1% decrease (**Figure 4**; available at www.jpeds.com). **Figure 5** shows an MRI from a representative patient of the current analysis that illustrates the decrease in cyst burden we observed.

Measurements of renal function (estimated glomerular filtration rate based on serum creatinine by the Schwartz formula in individuals <18 years of age, and the Modification of Diet in Renal Disease equation for individuals >18 years of age) were available in 10 of the 15 patients at the time of their initial and follow-up MRI. Renal function was normal in all patients for whom data were available at the initial MRI (mean estimated glomerular filtration rate, 131 ± 27.4 mL/min/1.73 m^2; median 134) as well as follow-up MRI (mean estimated glomerular filtration rate, 125 ± 17.5 mL/min/1.73 m^2; median, 121). Renal function values were not different between initial and follow-up MRI (Student *t* test; $P = .49$).

Discussion

We noted improvement in cyst burden in terms of total number of cysts, sum of cyst diameter, and total volume of cysts. The dramatic improvement in cystic renal disease in this cohort suggests that mTOR inhibitors could be useful to controlling renal cystic disease in patients with TSC. Limitations of the study, including retrospective design, small sample size, and nonstandardized mTOR inhibitor dosage and treatment duration, necessitate that our observations be confirmed in future studies that overcome these limitations. An additional limitation is the lack of a control population. Although it is possible that spontaneous improvement and resolution of the cysts could occur without mTOR inhibition, this is unlikely given the known natural history of renal cysts in tuberous sclerosis.[6,9,12] Finally, although the 2 evaluators were not blinded to the study timepoint, we believe that this potential source of bias was mitigated by the selection of objective methods of cyst assessment such as the number of cysts, the sum of maximum cyst diameter, the measurement of fluid attenuation pixels, and the fact that the reviewers were blinded to each other's results. The strong correlation of findings between evaluators and the unequivocal results support our contention that the reduction in cyst burden is a real finding. This report provides first evidence of proof of concept that they may be effective in this regard and worthy of continued investigation for this significant aspect of TSC.

mTOR inhibitors are an effective therapy for various manifestations of TSC, including subependymal giant cell astrocytomas, angiomyolipomas, angiofibromas, cardiac rhabdomyomas, and seizures. mTOR inhibitors are generally well-tolerated. From a renal standpoint, mTOR inhibitors can cause proteinuria owing to glomerular dysfunction,[26] but this rarely requires dosage adjustment or discontinuation of treatment in TSC.

mTOR inhibition has shown beneficial effects in preclinical animal models of polycystic kidney disease. There is also evidence of mTOR activation in a subset of renal cyst-lining epithelial cells in ADPKD.[27-29] Previous clinical trials in human ADPKD patients have failed to show a beneficial effect of these agents to reduce renal cyst burden or improve clinical outcome.[22-24] One possible reason for this discrepancy between preclinical and clinical observations is that some but not all ADPKD cysts exhibit mTOR activation,[28] suggesting that this pathway may only be active during certain stages of ADPKD cyst development or that full/direct mTOR activation may not result from loss of function of ADPKD proteins in the same manner as would occur with loss of TSC1 or TSC2 protein function. In addition, the patients studied in these trials had late-stage ADPKD, which may have precluded observable clinical benefits that might only occur early in the disease. Indeed, it has been suggested that mTOR inhibition may benefit ADPKD patients in a preventative fashion if administered early.[24,30] Epithelial cells lining TSC cysts are hypertrophic and hyperplastic with strongly eosinophilic cytoplasm, owing to high protein content[31] and can be positive for TSC-specific markers such

as HMB-45.[12] These features are unique to TSC renal cystic epithelia, and support a model in which TSC-associated cystogenesis is induced by loss of heterozygosity of *TSC1* or *TSC2* leading to direct, aberrant activation of mTOR signaling in renal epithelial cells that may represent a molecularly distinct process from the mTOR-associated changes observed in ADPKD. For this reason, the observation of a beneficial therapeutic effect of mTOR inhibition in TSC renal cystic disease, although novel and distinct from that of ADPKD, is not entirely unexpected. Recent insights into cross-talk between mTOR signaling and polycystin-1 (1 of the proteins mutated in and associated with ADPKD) suggest that mTOR inhibition may be particularly beneficial for patients with CGS.[32]

As many as 50% of persons with TSC develop multifocal renal cysts, even in the absence of a CGS mutation. Renal cysts in this context are typically progressive,[9] and may result in significant chronic kidney disease, particularly with comorbid hypertension, overuse of nonsteroidal anti-inflammatory drugs, angiomyolipomas, or hemorrhage, which occur frequently.[33] Our patients were asymptomatic from a renal standpoint and tended to be younger, in contrast with studies of mTOR inhibitors in patients with ADPKD. It remains unknown if TSC-related renal cystic disease might respond better if therapy is begun at an earlier age. As mentioned, the findings of the present study warrant confirmation and further evaluation in prospective clinical trials in both patients with TSC and patients with CGS. Improvement in TSC-related renal cystic disease could delay the loss of renal function attendant to TSC[34] and represent a significant additional benefit of mTOR inhibition in this disease. ■

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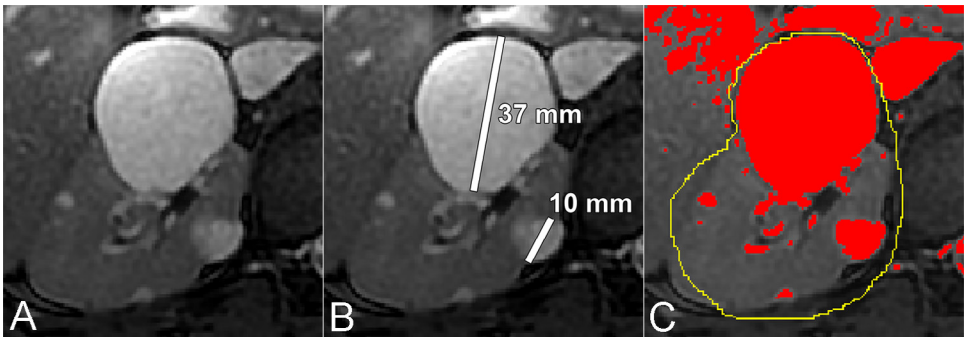

Figure 1. Example of image analysis for a 10-year-old female with tuberous sclerosis. **A,** Sample axial MRI image with multiple right renal cysts. **B,** Axial measurements of 2 of the cysts. **C**, Image thresholding (*red overlay*) with manual region of interest placement (*yellow line*) for cyst volume measurement.

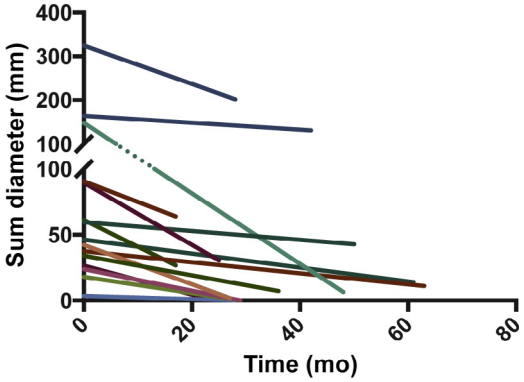

Figure 2. Change in the sum maximum diameter of the 20 largest cysts (maximum of 10 per kidney) for each patient over time. Each line represents 1 patient. The scale on the *y* axis is discontinuous to depict the broad range of values.

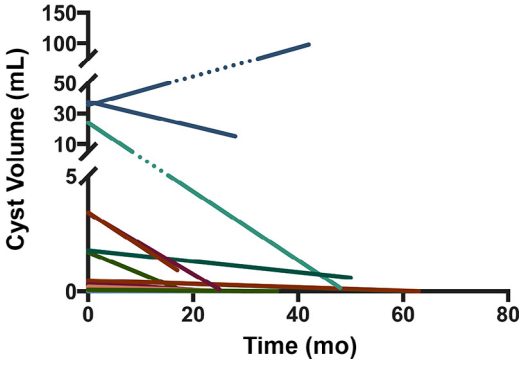

Figure 3. Change in the total cyst volume for each patient over time. Each line represents 1 patient. The scale on the *y* axis is discontinuous to depict the broad range of values.

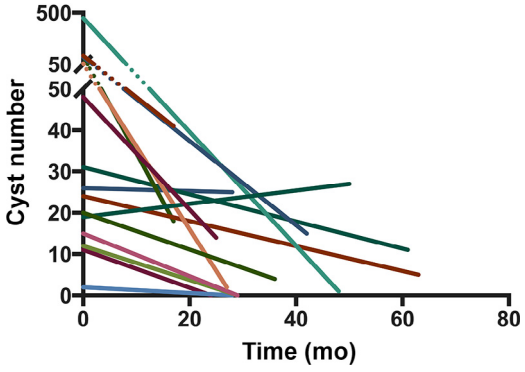

Figure 4. Change in the total number of cysts for each patient over time. Each line represents 1 patient. The scale on the *y* axis is discontinuous to depict the broad range of values.

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Table I. Patient information

Patients	Sex	Age (y)	Race	Genetic analysis	Means of diagnosis	Renal diagnosis	mTOR inhibitor	Indication for use	Age at start of treatment (y)	Timing of treatment
1	Male	5.9	White	*TSC2*- T5027C (pL1676P)	Infantile spasms	Cysts and AMLs	Everolimus	SEGA	3.5	Ongoing
2	Female	20.4	White	Not tested	Prenatally with cardiac rhabdomyoma	Cysts and AMLs	Everolimus	SEGA	16.8	Ongoing
3	Male	10.7	White	*TSC2*- C5024T	Unknown	Cysts	Everolimus	SEGA	4.4	Ongoing
4	Female	21.3	White	*TSC2*- splice site mutation, IVS22+1 G > C	Infancy with cardiac rhabdomyoma	Cysts and AMLs	Everolimus, Sirolimus	SEGA, facial angiofibromas, AMLs, epilepsy	15.5	Ongoing
5	Female	15.7	White	*TSC2*- C3442T	Infancy with hypopigmented macule	Cysts and AMLs	Everolimus	SEGA	10.2	Ongoing
6	Female	7.8	Asian	Not tested	Seizures in infancy	Cysts and AMLs	Everolimus	Epilepsy, retinal lesion	4.9	Ongoing
7	Male	5.4	White	Unknown	Seizures in infancy	Cysts and AMLs	Everolimus	SEGA, epilepsy	1.1	Ongoing
8	Female	6.7	White	*TSC1*- C1303T	Seizures age 2	Cysts	Everolimus	SEGA	3.2	Ongoing
9	Female	15.5	White	Not tested	Unknown	Cysts and AMLs	everolimus	SEGA, AMLs	11.2	Ongoing
10	Male	6.7	Hispanic	Not tested	Cardiac rhabdomyomas at birth	Cysts and AMLs	Everolimus	Epilepsy	3.7	Ongoing
11	Female	13.5	White	Unknown	Unknown	Cysts	Sirolimus	SEGA, epilepsy	5.8	Ongoing
12	Female	12.3	White	*TSC2*- C5024T	Unknown	Cysts and AMLs	Everolimus	SEGA, epilepsy	9.0	Ongoing
13	Male	4.4	White	Not tested	Infantile spasms	Cysts	Everolimus	Epilepsy	2.1	Ongoing
14	Female	10.5	White	Unknown	Infantile spasms	Cysts	Everolimus	SEGA	4.5	Ongoing
15	Female	25.8	White	*TSC2*- deletion, exons 3-5	Cardiac rhabdomyomas in infancy	Cysts and AMLs	Everolimus	AMLs	20.3	Ongoing

AML, angiomyolipoma; *SEGA*, Subependymal giant cell astrocytoma.

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