Renal manifestations of tuberous sclerosis complex: Incidence, prognosis, and predictive factors

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Tuberous sclerosis complex is a genetic disorder characterized by hamartomatous lesions in multiple organs, frequently involving the kidney. We conducted a retrospective review of the clinical and radiographic records of 167 patients with tuberous sclerosis to determine the frequency of renal disease, the likelihood of significant renal morbidity, and the effects of genotype (*TSC1* vs *TSC2*) and gender on renal phenotype. Renal lesions were seen in 57.5% of patients. Of these, angiomyolipoma (AML) occurred in 85.4%, cysts in 44.8%, and renal cell carcinoma in 4.2%. Both AML and cysts were significantly more common and more numerous in *TSC2* than in *TSC1*. AML was significantly more common in female than in male patients, but cysts showed no correlation with gender. Eleven patients developed renal abnormalities during their care in this practice at an average age of onset of 11.3 years (range 3.8–23 years). The frequency and number of renal lesions were positively correlated with age. Interventions, including arterial embolization and nephrectomy, were performed in 11 (6.6%) patients. Among female patients with lymphangioleiomyomatosis, renal AML was universally present. Our findings confirm a high rate of renal involvement; a low rate of serious complications; significant associations between renal involvement, genotype, and gender; and a significant association between renal and pulmonary involvement in female patients.

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Tuberous sclerosis complex (TSC) is a genetic disorder characterized by the growth of dysgenic lesions in multiple organs including the brain, skin, kidney, heart, lungs, and retina. TSC arises from inactivating mutations of either *TSC1* (chromosome locus 9q34.3) or *TSC2* (16p13.3), which encode hamartin and tuberin, respectively. These proteins are believed to function as tumor suppressors by forming a complex that regulates cellular proliferation.[1] Loss of heterozygosity at the *TSC2* locus has been observed in the renal and pulmonary tumors associated with TSC,[2–5] suggesting that tumor development follows loss of the functional allele. Whereas mutations in *TSC1* and *TSC2* impact the same organ systems, *TSC2* mutations tend to result in a more severe clinical profile, including more acute renal involvement.[6,7]

Renal manifestations occur with a high frequency and a wide range of severity in TSC.[8–10] Estimated rates of involvement range from 48[11] to 80%,[10] with at least two large studies reporting an incidence between 60 and 75%.[12,13] Although the prevalence of renal findings in TSC is well known, questions regarding age of onset, tumor growth rate, frequency of clinical significance, and the effects of gender and genotype remain unanswered.

The two renal pathologies most commonly seen in TSC are angiomyolipomas (AMLs) and cysts. AMLs are more prevalent, with a frequency in TSC of 34–80%.[10,12–14] Generally seen in the cortex, AMLs are composed of adipocytes, abnormal vasculature, and smooth muscle cells, the proportions of which may vary even across lesions in a single patient.[8] The potential complications of AML include hemorrhage, the risk of which increases with the size and vascularity of the lesion, and mass effects, which can cause discomfort or pain and can compromise renal function by compressing urine outflow and/or distorting normal renal parenchyma. Complications are generally treated by angiographic arterial embolization or surgical extirpation, with nephron-sparing procedures preferred to radical nephrectomy.[8,15] Some researchers have suggested that certain AML variants are capable of metastatic growth,[2,16] but this proposal remains controversial.

Renal AML in TSC is also associated with the development of pulmonary lymphangioleimyomatosis (LAM), a

progressive cystic disease of the lungs affecting women almost exclusively. AML is observed in approximately 88% of patients with TSC and LAM[17] and AML usually predates the onset of pulmonary disease. However, the causes underlying the association are poorly understood.

Renal cysts are observed with a frequency of 14–32% in the TSC population,[12–14] and exhibit two modes of presentation. In the more common presentation, cysts are single or multiple small lesions that are uniform in histology and rarely symptomatic.[10] Less commonly, TSC co-exists with polycystic kidney disease, which carries a poor prognosis for renal survival. These cysts are numerous, large, and frequently symptomatic. The *TSC2* locus is adjacent to one of the genes for polycystic kidney disease (*PKD1*), and contiguous deletions can produce both phenotypes.[18] The relationship between non-polycystic kidney disease renal cysts and TSC genotype has not been well studied.

Anecdotal evidence suggests that renal cell carcinoma occurs more frequently and at a younger age in TSC than in the general population,[13,19] although this has not been confirmed.

This study investigates the epidemiology and sequellae of renal disease in a cohort of 170 patients with TSC, and examines whether genotype and gender predict renal phenotype.

RESULTS

Subjects ranged in age from 1 month to 59 years (mean 15.7 years, s.d. 14.4). There was no significant difference in mean age between male and female patients.

Mutational analysis had been performed on 136/167 patients (81.4%): 36 had mutations in *TSC1* (26.4%), 75 had mutations in *TSC2* (55.1%), and 23 patients who met the clinical criteria for TSC had no identified mutation (NIM) by standard genotyping (16.9%). Two patients had somatic mosaicism of the *TSC2* gene mutation and were excluded from further analysis. One patient had disease-causing mutations in both TSC genes. Given the greater severity of the *TSC2* phenotype,[6,7,20] this patient was included in the *TSC2* subset. Two of the NIM patients had *TSC2* polymorphisms known to be benign. The greater prevalence of *TSC2* over *TSC1* mutations was consistent with prior studies, as was the proportion of patients with NIM.[6,20] There was no significant relationship between genotype and gender; however, patients with *TSC2* were younger on average than patients with *TSC1* ($P = 0.022$; Table 1).

On average, between two and three radiology reports were available per patient. Of 167 patients total, ultrasound had been performed in 148 (88.6%), magnetic resonance imaging (MRI) in 29 (17.4%), and computed tomographic (CT) in 29 (17.4%). Forty-one patients had undergone more than one type of imaging. Twelve patients lacked radiology reports, but were able to report their imaging findings to us in sufficient detail to include them in analyses.

Renal lesions of any type were found in 96/167 (57.5%) patients. Of the 96 renal patients, 82 (85.4%) had AML, 43 (44.8%) had cysts (of whom 30 had co-existing AML), four (4.2%) patients had renal cell carcinoma, and three (3.1%) had ambiguous diagnoses (i.e. renal cell carcinoma vs fat-poor AML) in addition to known AML. Of the 30 female patients aged 16 years and older, cystic lesions on pulmonary CT consistent with LAM were observed in 10 (33.3%).

Six patients had a history of hemorrhagic episodes, detected on imaging or by hematuria – five associated with AMLs and one with cysts. A total of 11 patients underwent invasive procedures, either for hemorrhagic AML or suspected renal cell carcinoma. Five patients underwent selective arterial embolization (mean age 23.6 years, range 5.8–37 years). Nine patients, including three who also had embolization, underwent a nephrectomy (mean age 24 years, range 6–37 years). One female patient with *TSC2* had progressive renal cell carcinoma confirmed by biopsy at our institution, and died at the age of 28 years. No patient progressed to end-stage renal disease while in our care.

A multiple regression analysis revealed that genotype had a significant effect on renal phenotype, when gender and age were controlled (Table 1). AMLs were significantly more frequent ($P < 0.001$), more numerous ($P < 0.001$), and more likely to be bilateral ($P < 0.001$) in *TSC2* than *TSC1*. Similarly, cysts were more frequent ($P = 0.025$), more numerous ($P = 0.005$), and more likely to be bilateral ($P = 0.047$) in *TSC2* than *TSC1*. Patients with *TSC2* were also more likely to have both lesion types ($P = 0.005$). Patients with NIM appeared to have more extensive AML involvement than patients with *TSC1*, but there were no differences in the incidence or severity of cysts. Overall, patients with NIM had renal phenotypes that more closely resembled those of *TSC2* than of *TSC1*, but they exhibited fewer AMLs and a lower rate of bilateral AML presentation than *TSC2* patients.

There were too few cases of renal cell carcinoma to test its correlation with gender or genotype. Of the four patients with confirmed renal cell carcinoma, one had *TSC1*, two had *TSC2*, and one had not been genotyped. Of the 11 patients who have undergone embolization or nephrectomy for a renal mass, three had not been tested, one had a mutation in *TSC1*, five had mutations in *TSC2*, and two had NIM. There were too few cases of invasive intervention to determine whether genotype influences the likelihood of needing these procedures.

McNemar's test was performed to test the comparative incidence of AMLs and cysts with respect to genotype. There was no significant difference in the frequencies of these two lesions in the NIM or *TSC1* genotypes. In *TSC2*, however, AMLs were significantly more common that cysts ($P < 0.001$).

AMLs were significantly more common in female than in male patients ($P = 0.006$), even when genotype and age were controlled, but the development of cysts was independent of gender (Table 2). Female patients were also more likely to have bilateral AMLs ($P = 0.032$) and a higher number of AMLs ($P = 0.014$), whereas cysts showed no analogous effect.

Table 1 | Genetic effects on renal involvement

	TSC1 patients (*N*=36)	*TSC2* patients (*N*=75)	NIM patients (*N*=23)	*P*-values *TSC1* vs *TSC2*	*P*-values *TSC1* vs NIM	*P*-values *TSC2* vs NIM
Male	14 (38.9%)	43 (57.3%)	11 (47.8%)	NS	NS	NS
Age[a]	18.8±15.8 (1.8–59.1)	12.6±11.7 (0.1–49.9)	14.8±14.2 (1.8–49.7)	0.022	NS	NS
AML involvement						
Frequency	8 (22.2%)	45 (60%)	11 (47.8%)	<0.001	0.006	NS
Number						
1	5 (62.5%)	1 (2.2%)	2 (18.2%)	<0.001	0.002	0.031
2–4	2 (25%)	1 (2.2%)	0	<0.001	0.001	—
>4	1 (12.5%)	43 (95.6%)	9 (81.8%)	—	—	0.023
				—	—	—
Bilateral lesions	2 (25%)	44 (97.8%)	9 (81.8%)			
Cystic involvement						
Frequency	6 (16.7%)	23 (30.7%)	6 (26.1%)	0.025	NS	NS
Number[b]						
1	4 (66.7%)	7 (30.4%)	2 (33.3%)	0.005	NS	NS
2–4	0	2 (8.7%)	0	0.047	NS	NS
>4	1 (16.7%)	13 (56.5%)	4 (66.7%)			
Bilateral lesions	1 (16.7%)	12 (52.2%)	4 (66.7%)			
AML and cysts[c]	2 (5.6%)	19 (25.3%)	3 (13.0%)	0.005	NS	NS
RCC	1 (2.8%)	2 (2.7%)	0	NS	NS	NS
Complications/interventions						
Hemorrhage	0	3 (4%)	1 (4.3%)	NS	NS	NS
SAE	0	3 (4%)	1 (4.3%)	NS	NS	NS
Nephrectomy	1 (2.8%)	3 (4%)	2 (8.7%)	NS	NS	NS
LAM[d]	1/9 (11.1%)	6/10 (60.0%)	2/4 (50.0%)	0.038	NS	NS

AML, angiomyolipoma; LAM, lymphangioleiomyomatosis; NIM, no identified mutation; NS, nonsignificant; RCC, renal cell carcinoma; SAE, selective arterial embolization; TSC, tuberous sclerosis complex.
Gender vs genotype was assessed with the χ^2 test; age vs genotype was assessed with Student's *t*-test; LAM vs genotype was assessed with Fisher's exact test. All other variables were tested for differences between genotypes with a multiple logistic regression, controlling for age and gender. Pairwise comparisons of *TSC1*, *TSC2*, and NIM were performed.
[a]Age on most recent renal study. Mean±s.d. (range).
[b]The number of cysts was unavailable for one *TSC1* patient and one *TSC2* patient with known cystic involvement.
[c]Patients with both lesions types were compared to patients with either lesion type or neither.
[d]Including only females of age ⩾16 after Dabora *et al.*[6] *P*-values were derived from Fisher's exact test.

Also, female patients were more likely than male patients to have both AMLs and cysts ($P=0.020$).

When gender and genotype were controlled, age was positively correlated with the presence, number, and bilaterality of both AMLs and cysts, and with the likelihood of having both lesion types (Table 2). The youngest patient to exhibit renal findings was a 1-year-old *TSC2* male patient with bilateral cysts. Twelve patients in whom initial imaging results were normal developed abnormal renal findings during their care in our practice, at an average age of 11.1 years and a range of 3.8–23 years. Three patients developed abnormal findings after the age of 20 years. There was insufficient data to identify a 'plateau age' after which renal lesions do not appear, nor to calculate meaningful rates of progression. The most rapid growth of AMLs in this cohort occurred in an asymptomatic *TSC2* female patient whose <5-mm bilateral AMLs at 13 years of age grew within 18 months to three large bilateral masses whose maximal dimensions were 6.2, 6.0, and 3.2 cm, respectively. The first imaging modality was ultrasound; the second was MRI.

Of the 30 female patients aged 16 years and older, 10 (33.3%) had cystic lesions on pulmonary CT consistent with LAM (Table 1). Of the 10 patients with LAM, six had *TSC2*, one had *TSC1*, and two had NIM. LAM was significantly more common in female patients with *TSC2* than *TSC1* ($P=0.038$). All patients with LAM had co-existing renal AML, and the association between AML and LAM was significant (Fisher's exact test, $P=0.022$).

DISCUSSION

Consistent with prior studies, we observed renal involvement in a majority (57.5%) of individuals with TSC. Whereas the prevalence of renal lesions in TSC is well known, little is known about the variables that predict renal phenotype. A better understanding of these factors will improve management of the disease and provide insight into the pathogenesis

Table 2 | Effects of gender and age on renal involvement

	Male (*N*=68)	Female (*N*=66)	*P*-values M vs F	*P*-values Age
AML involvement				
Frequency	25 (36.8%)	39 (59.1%)	0.006	<0.001
Number				
1	1 (4.0%)	7 (17.9%)	0.014	<0.001
2–4	1 (4.0%)	2 (5.1%)		
>4	23 (92.0%)	30 (76.9%)		
Bilateral lesions	23 (92.0%)	32 (82.1%)	0.032	<0.001
Cystic involvement				
Frequency	15 (22.0%)	20 (30.3%)	NS	0.040
Number				
1	4 (26.7%)	9 (45.0%)	NS	0.005
2–4	2 (13.3%)	0		
>4	8 (53.3%)	10 (50.0%)		
Bilateral lesions	8 (53.3%)	9 (45.0%)	NS	0.037
AML and cysts	7 (10.3%)	17 (25.8%)	0.020	0.027

AML, angiomyolipoma; F, female; M, male.
Type III *P*-values were determined from a multiple logistic regression with terms for genotype, age, and gender. Includes only patients who had undergone genetic testing. Both AMLs and cysts increased in frequency, number, and bilaterality with age. *P*-values show the effect of an increase in age on the likelihood of having the symptom described. The number of cysts was unavailable for one male and one female patient with known cystic involvement.

of TSC-related tumors both of the kidney and of other organs.

Effects of genotype

TSC genotype is a strong predictor of renal involvement. Patients with *TSC2* mutations exhibited a higher incidence and severity of both AMLs and cysts, compared to patients with *TSC1* (Table 1). These results demonstrate the utility of genetic testing in the clinical care of patients with TSC, as renal outcomes can be more accurately predicted if mutational results are known. Although severe renal disease including renal cell carcinoma can occur in *TSC1*, patients with *TSC1* mutations are overall significantly less likely to exhibit renal manifestations than those with *TSC2*. A larger sample is needed to determine whether there is an effect of genotype on the development of renal cell carcinoma and the likelihood of needing invasive intervention.

TSC patients who have NIM generally exhibit an overall syndromic phenotype that is less severe than patients with known mutations, especially with respect to cognitive and seizure outcomes.[6,7] However, this phenomenon is not recapitulated in the renal phenotype. Sancak *et al.*[7] observed that AMLs were more common in patients with NIM than in those with *TSC1*. Dabora *et al.*[6] observed a higher grade of AML involvement in patients with NIM than in *TSC1* and no difference between NIM and *TSC2*. Similarly, our findings suggest that the renal phenotype of patients with NIM more closely resembles that of *TSC2* than *TSC1*. This suggests that NIM patients may have a transcriptional, translational, or post-translational defect in *TSC2*, and that these patients warrant closer examination of their *TSC2* sequence and protein activity.

It is worth noting that patients with *TSC2* mutations were younger on average than patients with *TSC1* mutations. Given that *TSC2* mutations often result in a more severe clinical profile, it is likely that patients with *TSC2* come to medical attention at a younger age.

Effects of gender

The relationship between gender and renal phenotype was significant only for AML involvement (Table 2). AMLs were more common and more numerous in female patients, after controlling for age and genotype. Sporadic AML is known to exhibit predominance in female patients,[8,15] an effect that has been attributed to the presence of estrogen and progesterone receptors on the tumors.[8] Other studies of TSC have suggested that AMLs may show a greater propensity for growth in female patients.[10,14] Further study is needed to determine whether hormonal modulation accounts for the difference in AML manifestations between female and male patients.

Age of onset and rate of growth

Our data show a significant association between increasing age and the incidence and number of both AMLs and cysts. This association was independent of the effects of gender and genotype and confirms prior observations that both lesion types increase in frequency with age.[21] These results highlight the importance of following patients with TSC for renal involvement throughout their adult lives.

Other studies have estimated the average age at onset of renal abnormalities to be between 7.2 and 9.2 years for AMLs [10,21] and 9 years for cysts.[21] Our results suggest a later onset (average 11.1 years). However, retrospective studies may overestimate age of onset, as patients with normal findings are not followed as frequently as those with abnormal ones. Prior studies have suggested that cysts may be present at birth, whereas AMLs begin appearing in the first year of life.[10] It is often assumed that patients with renal involvement will develop lesions by adolescence; however, it is unknown whether there is a plateau age after which renal lesions are unlikely to develop. Little is known about the growth rate of AMLs in TSC or the likelihood that they will remain stable over time.

Although our results support the notion that renal involvement usually develops by adolescence, two cases from this population offer instructive and important examples to the contrary. In one case, a female patient who had had a normal renal ultrasound at age 21 years was found on routine ultrasound at age 23 years to have a $3.6 \times 3.1 \times 3.9$cm solid mass suspicious for renal cell carcinoma. In another case, multiple small AMLs were detected on routine ultrasound in a 20-year-old male patient who had had a normal ultrasound at age 18 years. The sensitivity and subjectivity of ultrasound is such that lesions are sometimes missed on a single study. However, these examples demonstrate the critical importance of obtaining follow-up renal imaging for patients with TSC throughout adulthood, even if scans

are normal through adolescence. We cannot rule out the possibility that renal lesions may develop in adulthood without a better understanding of the molecular mechanisms by which they form.

Given the retrospective nature of the study, we were unable to estimate tumor growth rate. Measurements of lesions size were hindered by the use of different imaging modalities on consecutive scans and by the lack of size estimates in many radiology reports. Comparisons across different modalities are complicated by differences in power and resolution, and the user-dependent nature of ultrasound compared to the more objective nature of CT and MRI. To our knowledge, there are no large-scale studies indicating that one modality provides measurably better detection than others in TSC.

More studies are needed to determine optimal surveillance protocols for renal imaging in TSC. We currently recommend that pediatric TSC patients have a baseline renal ultrasound before 5 years of age. If results are normal, follow-up ultrasound should be obtained every 2–3 years. If results show AMLs or cysts, we recommend follow-up ultrasound yearly thereafter. If results are suspicious for renal cell carcinoma, we recommend MRI for re-evaluation and follow-up imaging at six-month intervals or more frequently if clinically indicated. The case of rapid growth in multiple AMLs in a patient under 15 years of age underscores the importance of yearly imaging in patients with known renal involvement.

Differentiating AML from renal cell carcinoma

Renal cell carcinoma has been observed in the TSC population in children as young as 2 years of age,[22] but debate continues over whether TSC mutations increase susceptibility to renal cell carcinoma[8,23] and whether TSC-related AML can progress to carcinoma.[9] Some authors have suggested that certain variants of AML are capable of metastatic growth resembling carcinoma;[2,16] however, this issue remains highly contested.

A persistent problem in diagnosing renal masses in TSC is the difficulty in distinguishing fat-poor AML from renal cell carcinoma on imaging. In this cohort, there were four pathologically confirmed cases of renal cell carcinoma. There were six additional cases of renal masses that could not be differentiated between AML and carcinoma on imaging, constituting a significant management dilemma. Biopsy is often discouraged, as it can cause highly vascular AMLs to hemorrhage and can scatter malignant carcinoma cells. Moreover, ambiguous lesions often exhibit ambiguous pathology. Nephrectomy must be undertaken with caution in TSC, as patients are predisposed to developing additional masses in the remaining kidney.

This issue is of significant concern in the treatment of renal masses in TSC. A clearer understanding of the natural history of AML is urgently needed. It will also be essential to determine the accuracy of radiographic diagnoses in order to reduce the reliance on surgical intervention.

Symptomatic complications

Our results suggest that renal lesions in TSC are infrequently symptomatic and rarely fatal. Nine (9.4%) of 96 patients with known renal lesions had undergone biopsy or nephrectomy for AML or renal cell carcinoma. Six (6.3%) had hemorrhagic episodes. One patient died of renal cell carcinoma. None had end-stage renal disease while being followed in this practice. Therefore, we emphasize that, although close follow-up is critical for all patients with renal involvement, the rate of significant renal morbidity in the overall TSC population is low.

Coincidence of AML and LAM

The association between AML and LAM in TSC is well known.[8,17,24–26] LAM is characterized by the proliferation of abnormal smooth muscle cells in the interstitial spaces of the lung, and affects female subjects almost exclusively.[8,27,25] Renal AML is seen in approximately 88% of TSC-related LAM and 30% of sporadic LAM.[28] Despite their co-occurrence, the relationship between these two pathologies is not well understood. One hypothesis suggests that both diseases result from aberrant function of the *TSC2* gene product in separate organs.[24,26] Loss of heterozygosity in *TSC2* has been observed in both sporadic and TSC-related LAM [5,28] and re-expression of the *TSC2* gene product appears to block the invasive migration of LAM cells *in vitro*.[26] These observations are also consistent with a second hypothesis suggesting that LAM arises through the non-malignant metastasis of AML cells from the kidney. This view is supported by evidence that LAM can recur following pulmonary transplant, exhibiting cells that are both patient- and donor-derived, and that women with larger AMLs are more likely to have LAM.[27,25] Furthermore, identical loss of heterozygosity loci have been observed in AML and LAM cells in patients with sporadic disease, suggesting a common cellular origin.[27]

In this cohort, all 10 of the female patients who exhibited pulmonary LAM also exhibited renal AML. This significant correlation ($P = 0.022$) suggests that AML is co-requisite with LAM and supports the hypothesis of non-malignant metastasis. Whereas LAM can occur in any TSC genotype, it is most common in *TSC2*, possibly owing to the predominance of AML in *TSC2*. Given that LAM was observed in 10/30 (33%) female patients over the age of 16 years, we recommend that all female patients with TSC be followed throughout adulthood for pulmonary involvement.

Conclusion

Renal involvement is common in TSC, but symptomatic complications are rare. Genotype, gender, and increasing age were shown to have significant effects on renal manifestations. The severity of renal involvement is markedly increased in *TSC2* compared to *TSC1*, female subjects have more extensive AML involvement than male subjects, and both the frequency and number of AMLs and cysts are positively correlated with age. Among patients with LAM, AML was

universally coincident, providing further evidence for a relationship between these pathologies. More studies are needed to determine the rate of growth and age of onset of renal lesions in TSC. Importantly, our findings indicate that renal lesions can develop after puberty, emphasizing the need to monitor patients with TSC for potential renal involvement throughout their adult lives.

MATERIALS AND METHODS

We conducted a retrospective review of the clinical and radiographic records of 203 patients who were followed for TSC by a single neurologist (EAT). Clinical consult notes were reviewed to ensure that all subjects met established diagnostic criteria for TSC.[29] Renal radiology reports were available for 170 patients; those without imaging records were excluded from the study. Three patients had a single renal mass that could not be differentiated between AML and renal cell carcinoma; these three were excluded from all analyses for a total sample size of 167 (79 female patients).

A total of 433 CT, MRI, and ultrasound reports were reviewed for descriptions of renal findings. When sequential scans were available, results were compared for evidence of lesion growth. Subjects' reported ages correspond to the date of their most recent renal scan. Radiology reports from chest CT scans on all female subjects over the age of 16 years (after Dabora *et al.*[6]) were reviewed for the presence of LAM. Mutational analyses with respect to *TSC1* and *TSC2* were completed at Athena Diagnostics (Worcester, MA, USA) and Massachusetts General Hospital (Boston, MA, USA).

The χ^2 test was used to assess the relationship between gender and genotype. Student's *t*-test was used to test age vs genotype. Fisher's exact test was used to test LAM vs genotype and AML vs LAM. The effect of genotype on the frequency, number, and bilaterality of AMLs and cysts was tested using a multiple regression (logistic for categorical variables, linear for continuous) controlling for age and gender. Pairwise comparisons of *TSC1*, *TSC2*, and NIM were performed. The effects of gender and increasing age on renal manifestations were assessed using a multiple regression, controlling for genotype. In all cases, statistical significance was defined as $P<0.05$.

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