Cyst-like tubers are associated with TSC2 and epilepsy in tuberous sclerosis complex

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ABSTRACT

Background: Tuberous sclerosis complex (TSC) is a genetic condition characterized by the presence of hamartomatous lesions in multiple organs, including tubers in the brain. The majority of patients with TSC have epilepsy. Some cortical tubers are epileptic foci, while others appear to be physiologically quiescent. It is unknown whether variations in tuber morphology may account for this difference. The objectives of this study were to determine the frequency of cyst-like tubers in patients with TSC, whether cyst-like tubers correlate with TSC genotype, and whether cyst-like cortical tubers are associated with a history of infantile spasms, epilepsy, or refractory epilepsy.

Methods: A retrospective chart review was performed of 173 patients with TSC. MRI images were evaluated for the presence of at least one cyst-like cortical tuber. Patient charts were then reviewed for genetic mutation, a history of infantile spasms, epilepsy, and epilepsy refractory to more than three medications.

Results: A total of 46% of patients had at least one cyst-like cortical tuber present on neuroimaging. Patients with a *TSC2* mutation were more likely to have a cyst-like tuber than patients with *TSC1* mutation ($p = 0.002$) or patients with no mutation identified ($p = 0.039$). Patients with at least one cyst-like cortical tuber were more likely to have a history of infantile spasms ($p = 0.00005$), epilepsy ($p = 0.0038$), and refractory epilepsy ($p = 0.0007$) than patients without a cyst-like cortical tuber.

Conclusion: Cyst-like cortical tubers are strongly associated with *TSC2* gene mutation and a more aggressive seizure phenotype in patients with tuberous sclerosis complex. ***Neurology*® 2009; 72:1165–1169**

GLOSSARY

CaMD = calmodulin binding domain; **CCD** = coiled-coil domain; **CI** = confidence interval; **ERM** = Ezrin–radixin–moesin; **FLAIR** = fluid-attenuated inversion recovery; **FSE** = fast spin-echo; **GAP** = GTPase activating protein; **LZD** = leucine zipper domain; **NEX** = number of excitations; **NMI** = no disease-associated mutation identified; **TAD** = transcription activating domain; **TE** = echo time; **TMD** = transmembrane domain; **TR** = repetition time; **TSC** = tuberous sclerosis complex.

Tuberous sclerosis complex (TSC) is a genetic disorder caused by germline mutations in the *TSC1* or *TSC2* genes and clinically characterized by the presence of hamartomatous lesions in multiple organs, including cortical tubers in the brain.[1-3] Protein products of the *TSC1* and *TSC2* genes form a heterodimer that inhibits the mammalian target of rapamycin complex 1 (mTORC1)[3] signaling cascade which has been implicated in tuber pathogenesis.[4,5]

Epilepsy affects approximately 85% of patients with TSC,[6,7] though the mechanisms underlying epileptogenesis in this disease remain unknown. The relationship between seizure severity, epilepsy surgery outcome, and cortical tuber burden has been closely examined but remains unclear.[8-15] Some cortical tubers are thought to be epileptic foci, while others appear to be physiologically quiescent. It is unknown whether variations in tuber morphology may account for this difference.

Although cortical tubers are primarily considered static congenital lesions, some tubers exhibit evolving cyst-like characteristics on MRI.[16-18] These lesions are called cyst-like since it is

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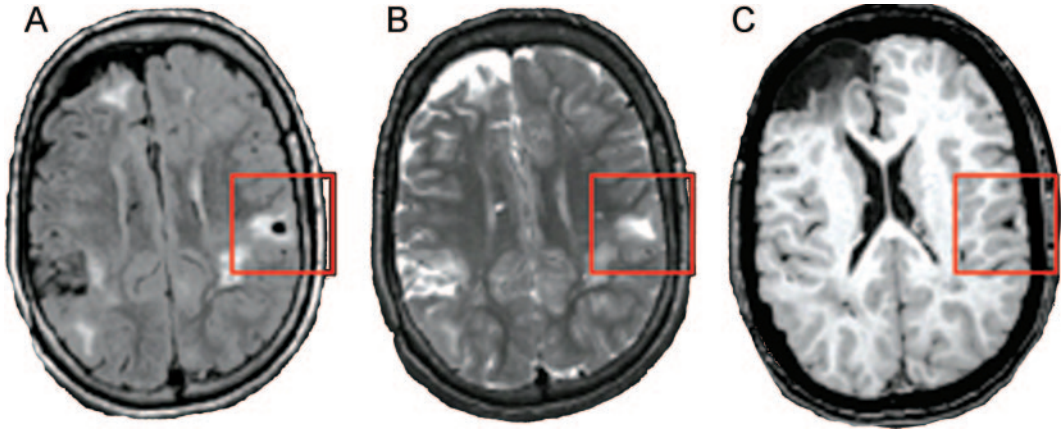
Figure 1 Example of a cyst-like tuber



This patient has a cyst-like cavity within a tuber in the left parietal lobe which is isointense to CSF on fluid-attenuated inversion recovery (A), T2 fast spin-echo (B), and T1 (C) images.

presumed that they lack the inner endothelial lining seen in true cysts. We sought to determine whether the presence of cyst-like cortical tubers correlates with TSC genotype, including mutations affecting known functional domains of the protein product. We further sought to characterize the frequency of cyst-like tubers in patients with TSC and evaluate whether the presence of these lesions correlates with seizure severity, including a history of infantile spasms, epilepsy, and refractory epilepsy.

METHODS We performed a retrospective chart review of patients seen at the Carol and James Herscot Center for Tuberous Sclerosis Complex at Massachusetts General Hospital between January 2002 and July 2007. Patients were included if they had definite clinical TSC and had brain MR images available for review. A total of 173 patients were included.

MR images meeting inclusion criteria were retrospectively reviewed by three study investigators blinded to clinical data (C.C., M.M., and P.M.), after consultation with a pediatric neuroradiologist. MRI scans were obtained on a 1.5- or 3.0-Tesla system (GE Signa, Madison, WI). Sequences obtained included spin-echo sagittal T1-wieghted (repetition time [TR]/echo time [TE]/number of excitations [NEX], 575/10/2), fast spin-echo (FSE) axial T2-weighted (TR/TE/NEX, 6,000/102/2), axial long inversion recovery-fluid level attenuated inversion recovery (fluid-attenuated inversion recovery [FLAIR]; TR/TE/NEX, 10,000/120/1), and three-dimensional spoiled gradient-recalled with steady-state acquisition (TR/TE/NEX/flip angle, 32/10/1/25). MRI slice thickness varied from 1.2 to 4 mm depending on the particular sequence. MRI sequences were evaluated for the presence or absence of at least one cyst-like cortical tuber. Brain lesions were identified in accordance with previous reports.[16,18] Cortical tubers were defined as areas of cortical gray matter distortion with decreased subcortical signal intensity on T1 and increased signal intensity on T2 and FLAIR sequences. Tubers demonstrating central FLAIR suppression, increase in FSE-T2 signal intensity, and relative decrease in T1 signal intensity were classified as cyst-like (figure 1). Cyst-like lesions not associated with surrounding cortical tuber were excluded.

Patient charts were retrospectively reviewed for genetic mutation, a history of infantile spasms, epilepsy, and epilepsy refractory to more than three anticonvulsant medications (not including treatments for infantile spasms). Genetic testing of the *TSC1* and *TSC2* genes included detection of large DNA deletions and rearrangements of the *TSC2* gene. Mutations were categorized by type (missense, inframe deletion, or insertion vs protein-truncating mutation) and location and whether or not they would affect the hamartin-tuberin interaction domains of the *TSC1* or *TSC2* gene products or the GTPase activating protein (GAP) domain of the *TSC2* gene product. Statistical analyses were performed using χ^2 analyses and Fisher exact test. All reported p values used two-tailed tests of significance with α set at 0.05.

The institutional review board of the Massachusetts General Hospital approved this study.

RESULTS The descriptive characteristics of the patients are shown in the table. Patients' age ranged from 2 months to 73 years at time of brain MRI (mean = 18 years 3 months, SD = 1 year 2 months, median = 13 years 0 months). A total of 95 patients were female and 78 patients were male. Cyst-like lesions were identified in 79 patients (46%). Patients younger than 18 years were more likely to have a cyst-like tuber ($p = 0.00006$, RR = 2.32, 95% confidence interval [CI] 1.46–3.71) than patients 18 years or older. There was no association between gender and the presence of a cyst-like lesion ($p = 0.45$).

A total of 29 patients in this study had *TSC1* mutations, 81 patients had *TSC2* mutations, 33 patients had no disease-associated mutation identified (NMI), and 30 patients were not tested. A total of 45 of 81 (56%) patients with an identified *TSC2* gene mutation, 6 of 29 (21%) patients with an identified *TSC1* gene mutation, and 11 of 33 (33%) patients with NMI had at least one cyst-like cortical tuber present on imaging. Patients with *TSC2* gene mutations

Table Descriptive characteristics of 173 patients with tuberous sclerosis complex evaluated for the presence of a cyst-like tuber

Characteristic	≥1 Cyst-like lesion, n (%)	No cyst-like lesion, n (%)
All patients	79/173 (46)	94/173 (54)
Gender		
Male	32/77 (42)	45/77 (58)
Female	47/96 (49)	49/96 (51)
Age, y		
≥18	15/61 (25)	46/61 (75)
<18	64/112* (57)	48/112 (43)
Gene mutation		
TSC1	6/29 (21)	23/29 (79)
TSC2	45/81* (56)	36/81 (44)
NMI	11/33 (33)	22/33 (67)
History of infantile spasms		
Yes	44/68* (65)	24/68 (35)
No	35/105 (33)	70/105 (67)
History of epilepsy		
Yes	73/144* (51)	71/144 (49)
No	6/29 (21)	23/29 (79)
History of refractory epilepsy		
Yes	63/114* (55)	51/114 (45)
No	16/59 (27)	43/59 (73)

*Significant relationship.

were more likely to have a cyst-like cortical tuber than patients with *TSC1* gene mutations ($p = 0.002$, RR = 2.7, 95% CI 1.28–5.62) or patients with NMI ($p = 0.039$, RR = 1.29, 95% CI 1.29–1.64). There was no relationship between the presence of a mutation affecting the hamartin-tuberin interaction domains of either the *TSC1* ($p = 1$) or *TSC2* gene products ($p = 0.77$) or the GAP domain on the *TSC2* gene ($p = 0.82$) and the presence of a cyst-like tuber (figure 2).

The presence of a cyst-like cortical tuber on neuroimaging was strongly associated with a seizure history. A total of 44 of 79 (57%) patients with at least one cyst-like tuber had a history of infantile spasms compared with only 24 of 94 (26%) patients without an identified cyst-like tuber ($p = 0.00005$, RR = 2.18, 95% CI 1.47–3.25). A total of 73 of 79 (92%) patients with at least one cyst-like cortical tuber had a history of epilepsy, compared with 71 of 94 (76%) patients without a cyst-like cortical tuber ($p = 0.0038$, RR = 1.22, 95% CI 1.07–1.40). Furthermore, 63 of 79 (80%) patients with at least one cyst-like cortical tuber had a history of refractory epilepsy, compared with 51 of 94 (54%) patients without a cyst-like cortical tuber ($p = 0.0007$, RR = 1.47, 95% CI 1.18–1.83).

Among the 92 patients without a *TSC2* mutation, 17 of 34 (50%) patients with at least one cyst-like tuber had a history of infantile spasms, compared with 6 of 58 (10%) patients without a cyst-like cortical tuber ($p = 0.0004$, RR = 4.83, 95% CI 2.11–11.07). Further subgroup analyses were not sufficiently powered to adequately evaluate.

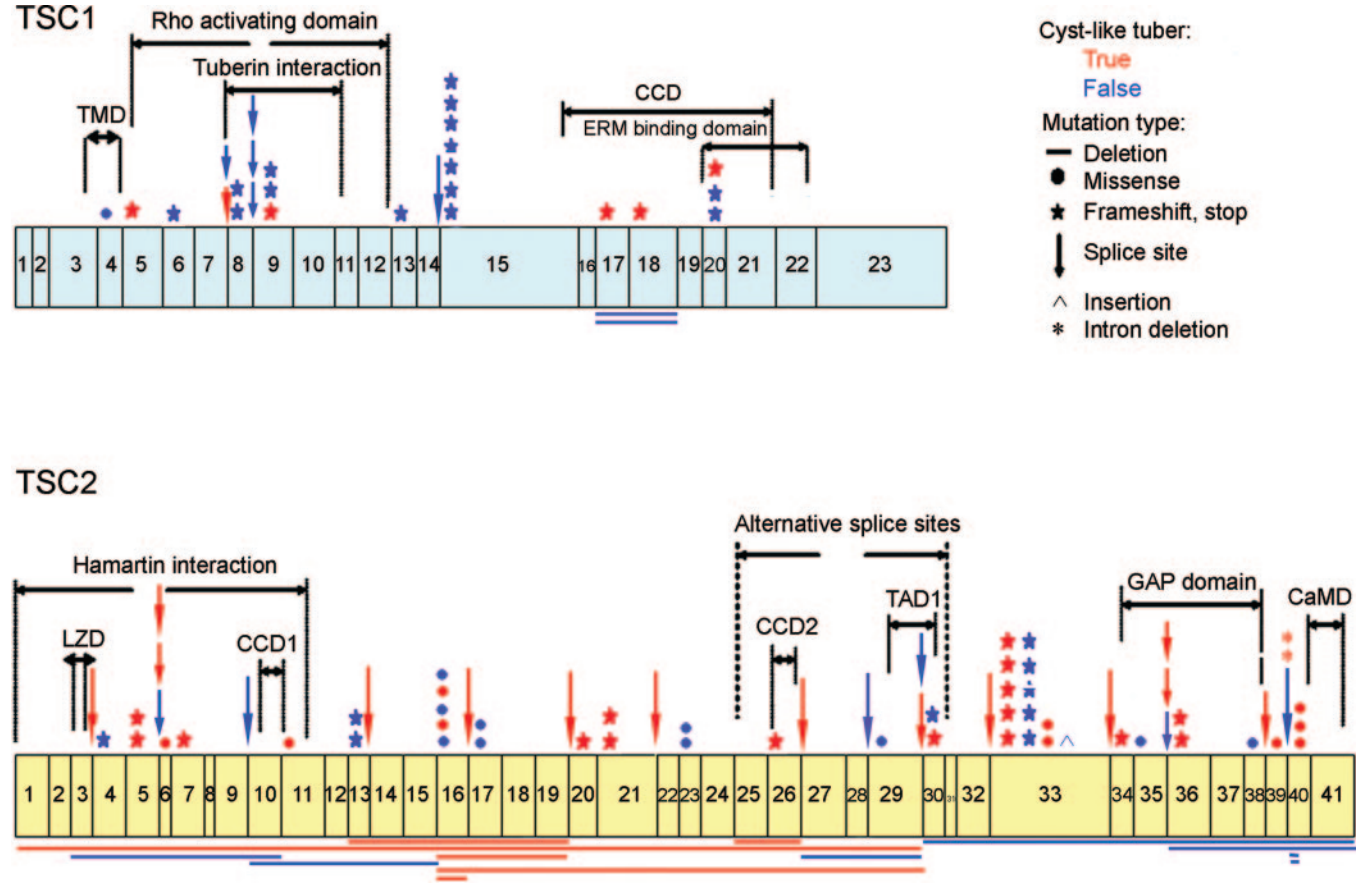
DISCUSSION Cortical tubers are areas of disrupted cortical architecture thought to be due to abnormal cellular proliferation, differentiation, or migration during fetal development. These lesions are composed of a variety of cell types, including dysplastic neurons and astroglia, giant neuroglial cells, disoriented pyramidal cells, and reactive astrocytes.[4,19,20] Tubers also exhibit abnormal cellular expression of excitatory glutamate receptors, altering their cortical network excitability.[20] The irregular architecture and physiology of cortical tubers and their adjacent cortex make them an attractive candidate for epileptic foci in TSC patients with epilepsy. Surgical resection of epileptic tubers and their adjacent cortex can result in cure for epilepsy.[21-23] However, determining whether and which cortical tubers are likely to be epileptogenic remains a challenge.

Earlier work has evaluated the prognostic significance of tuber burden on seizure history in TSC patients,[8-12,24] but tuber morphology has not been previously evaluated. We found that cyst-like cortical tubers are present in nearly half of TSC patients. Furthermore, we found a strong correlation between the presence of cyst-like cortical tubers and a history of infantile spasms, epilepsy, and severe epilepsy refractory to medical treatments.

The cyst-like tubers described here are located within cortical tubers and should be differentiated from previously described small cystic subcortical cavities commonly seen in patients with TSC. These cystic cavities are typically located in the deep white matter, often near the lateral ventricles. They usually are not associated with adjacent or surrounding signal abnormalities.[25]

The pathogenesis of cystic change in cortical tubers is unknown. Previous authors postulate that cyst-like lesions result from cellular degeneration of tubers,[3,19] disordered neuronal migration, or both.[16] Alternatively, these changes may be the result of accelerated apoptotic processes in altered neuronal and astroglial cells. Interestingly, we and others[13] have found that younger patients are more likely to have cyst-like cortical tubers than older patients. This finding may reflect a more aggressive subset of TSC, resulting from a sample bias in our clinic population. Alternatively, cyst-like lesions may become less apparent on MR imaging over time.

Figure 2 *TSC1* and *TSC2* gene exon maps of mutation type in 110 patients with known *TSC* gene mutations with and without cyst-like tubers



TMD = transmembrane domain; CCD = coiled-coil domain; ERM = Ezrin–radixin–moesin; LZD = leucine zipper domain; TAD = transcription activating domain; GAP = GTPase activating protein; CaMD = calmodulin binding domain.

Genotype–phenotype relationships are well-established in TSC with more severe multiorgan disease seen in patients with *TSC2* gene mutations. Patients with *TSC2* mutations exhibit higher frequency of seizures, moderate and severe mental retardation, subependymal nodules and cortical tubers, severe kidney involvement, dermatologic involvement, and pulmonary lymphangiomyomatosis than patients with *TSC1* mutations.[3,26,27] This work refines the *TSC2* phenotype to include a higher frequency of cyst-like cortical tubers. Previous studies have also reported possible correlations between the mutation type and neurologic phenotype.[27,28] We did not find a relationship between the predicted significance of mutation type and location on protein function and the presence of cyst-like tubers.

At this time, it is unknown whether cyst-like tubers have altered physiologic characteristics which may contribute to a more severe seizure phenotype or whether they are independent features seen in more malignant disease. Of note, even among patients without *TSC2* mutations, the presence of a cyst-like tuber was an independent risk factor for infantile spasms. Further work, including pathologic evaluation of these lesions, analysis of the relationship between cyst-like tuber burden on epilepsy outcome, determining whether cyst-like tubers have an electrographic correlate, and analysis of epilepsy surgery outcome targeting these lesions, needs to be done to determine if the anatomic differences identified on neuroimaging can contribute to or account for the variable epileptic profiles seen in patients with TSC.

AUTHOR CONTRIBUTIONS

Statistical analysis was performed by Dr. Catherine Chu-Shore.

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