Published in final edited form as:

Ann Hum Genet. 2009 March ; 73(2): 141–146. doi:10.1111/j.1469-1809.2008.00496.x.

Distinct clinical characteristics of Tuberous Sclerosis Complex patients with no mutation identified

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Abstract

Tuberous Sclerosis Complex (TSC) is a multi-system disorder that is highly variable in its clinical presentation. Current molecular diagnostic methods permit identification of mutations in either *TSC1* or *TSC2* in 75–85% of TSC patients. Here we examine the clinical characteristics of those TSC patients who have no mutation identified (NMI). A retrospective review of our patient population that had comprehensive testing for mutations in *TSC1/TSC2* identified 23/157 (15 %) that were NMI. NMI patients had a lower incidence of brain findings on imaging studies, neurological features, and renal findings than those with *TSC2* mutations. In contrast, NMI patients had a lower incidence of seizures than TSC patients with *TSC1* mutations, but had a higher incidence of both renal angiomyolipomas and pulmonary lymphangioleiomyomatosis. This distinct constellation of findings suggest that NMI patients may have a unique molecular pathogenesis, different from that seen in TSC patients with the usual mutations in *TSC1* and *TSC2*. We suggest that the mechanisms of disease in these patients include both mosaicism for a *TSC2* mutation, and unusual non-coding region mutations in *TSC2*.

Keywords

tuberous sclerosis complex; no mutation identified; mosaicism; lymphangioleiomyomatosis; angiomyolipoma

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder affecting 1/6000 live births, and is characterized by the growth of benign hamartomas in multiple organ systems (Crino et al. 2006). Commonly affected organs include the brain, skin, kidneys, eyes, heart, lungs and liver. TSC is also associated with significant behavioral problems, seizures and cognitive impairment. The phenotypic manifestations in affected persons vary greatly. In patients meeting standardized (Roach and Sparagana. 2004) clinical criteria for TSC, a disease causing mutation in either *TSC1* or *TSC2* is found in 75–85% of cases (Dabora et al. 2001; Ali et al. 2005; Sancak et al. 2005; Au et al. 2007).

Even though for most *TSC1* and *TSC2* mutations there have been no consistent genotype/phenotype correlations between specific mutation types and associated clinical manifestations (Janssen et al. 1990; Niida et al. 1999; Dabora et al. 2001; Humphrey et al. 2004; Ali et al. 2005; Au et al. 2007), many studies show that *TSC2* patients have overall

both a higher incidence and more severe features of TSC than patients with *TSC1* mutations, including more significant neurological, renal and other organ involvement (Jones et al. 1999; Dabora et al. 2001; Langkau et al. 2002; Sancak et al. 2005; Au et al. 2007). However there are also several missense mutations in *TSC2* which have been associated with distinctly mild clinical features, such that some family members bearing these *TSC2* missense mutations do not fulfill standard diagnostic criteria (Khare et al. 2001; O'Connor et al. 2003; Mayer et al. 2004; Jansen et al. 2006). In addition, TSC patients with deletions encompassing the *TSC2* and *PKD1* loci have a severe renal phenotype (Sampson et al. 1997).

TSC patients without confirmed disease-causing genetic mutations ('no mutation identified', NMI) have been reported as having milder phenotypes compared to those with *TSC2* mutations and similar phenotypes compared to those with *TSC1* mutations (Dabora et al. 2001; Sancak et al. 2005). Renal involvement is an exception; it is commonly seen in NMI patients and is more similar to the *TSC2* phenotype (Dabora et al. 2001; Sancak et al. 2005).

When patients undergo molecular testing for *TSC1/TSC2* mutations, and are found to be NMI, careproviders are at a loss to provide a satisfactory explanation as to how this might occur, and what it means in terms of disease severity (prognosis) and familial transmission. To provide further information on these important questions, we performed a comprehensive review of all patients seen in our clinic (The Herscot Center for TSC) at the Massachusetts General Hospital from 2002 to 2008 to identify NMI patients, and assess their clinical features relative to other TSC patients in whom mutations in *TSC1* and *TSC2* were identified.

Materials and Methods

A retrospective chart review of TSC patients evaluated at The Herscot Center for TSC at the Massachusetts General Hospital (MGH) between January 2002 and April 2008 was performed. The study was conducted with the approval of Partners Human Research Committee. 270 patients were seen who met standard clinical criteria for the diagnosis of TSC (Roach and Sparagana. 2004), with a mean age of 19.5 years, median of 13.6 years, and range 0.2 – 73.4 years. 219 of these TSC patients had molecular diagnostic analysis for mutations in *TSC1* or *TSC2*. The majority (148, 68%) of these patients had the Complete Tuberous Sclerosis Evaluation [TSC1, TSC2] performed at Athena Diagnostics (Worcester, MA); while 51 (23%) were analyzed in the lab of DJK (Dabora et al. 2001) and 20 (1%) were analyzed at the MGH. All positive findings by DJK were confirmed by the Massachusetts General Hospital DNA Diagnostic Laboratory. Beginning in October 2005 all tests performed at Athena included the TSC2 MLPA assay for large genomic deletions in *TSC2*. In addition, whenever possible patients who had been tested previously and had NMI were tested for TSC2 deletions once this test became available. Fifty patients were found to have disease-causing mutations in *TSC1*, and 106 in *TSC2* (including 18 TSC2 large genomic deletions). In one patient mutations were found in both *TSC1* and *TSC2*. Three patients were found to have TSC2 mosaicism. In 18 patients variants of uncertain clinical significance were found (4 in the *TSC1* gene and 14 in the *TSC2* gene). Eighteen patients were tested for TSC1/TSC2 mutations, but did not have analysis for TSC2 genomic deletions. Because we wished to study patients with 'pure' NMI, our NMI group consisted of the remaining 23 patients with no mutation identified, excluding a total of 36 patients who had genetic testing in whom no disease causing mutation was identified: 18 patients with variants of uncertain significance and 18 who did not have TSC2 large deletion testing.

For comparison with the NMI group, we included all patients with definite disease-causing mutations. In familial cases only the index patient for each family was included, excluding 10/50 (20%) *TSC1* cases and 12/106 (11%) *TSC2* cases.

Demographic, clinical and radiographic data were collected for all 157 *TSC1*, *TSC2* and NMI patients. Presence or absence of the following features was noted: seizures, infantile spasms, cognitive impairment (IQ or DQ ≤ 70 , or lack of age appropriate cognitive development as notated in neurological examination by ET), presence or absence of subependymal nodules (SEN), subependymal giant cell tumors (SGCT) and tubers according to radiological reports, skin involvement (presence of at least one of the following: two or more hypomelanotic macules, facial angiofibroma, shagreen patch, forehead plaque), kidney angiomyolipoma (AML) and cysts, and pulmonary lymphangioleiomyomatosis (LAM) involvement. Age at last clinical visit, and age at the time of the most recent radiographic evaluation for renal and chest features were also noted. Cardiac rhabdomyomas were not included in the analysis, since there was no reliable information for the majority of the NMI patients.

Statistical comparisons were performed using the STATPLUS add-in for Microsoft Excel 2000. Chi square (χ^2) tests were used to compare binary features and student's unpaired t-test was used to compare quantitative measures. Mc Nemar's test was used for matched pairs comparisons.

Results

We compared the phenotypes of 157 patients with clinical TSC from our cohort of 219 individuals who had undergone TSC gene mutational testing (see Methods for exclusions). Twenty-three patients were NMI (15%), 40 had *TSC1* mutations (25%), and 94 had *TSC2* mutations (60%) in the group that we compared in detail (see Table 1 for demographics).

Phenotypic comparisons

Multiple clinical features were significantly less frequent in NMI patients compared to those with *TSC2* mutations: seizures, infantile spasms, cognitive impairment, subependymal nodules, SGCT, tubers, renal cysts, and renal angiomyolipoma (Table 1). Interestingly, pulmonary LAM in adult women was somewhat higher in the NMI group (80% vs. 44%), but did not reach statistical significance due to the small number of adult women in the population.

Some clinical features were also significantly less frequent in NMI patients compared to those with *TSC1* mutations: seizures, and skin involvement (borderline at $p=0.058$) (Table 1). In contrast, both renal AMLs and pulmonary LAM were significantly more frequent in the NMI patients compared to *TSC1* patients.

Since renal features are known to be both age and gender dependent (Rakowski et al. 2006), we compared age matched NMI patients to *TSC1* and *TSC2* patients within each gender (Mc Nemar's test, pairs matching within a ± 2 year range, Table 2). NMI males had a lower incidence of AML and of renal cysts than males with *TSC2* mutations. Female patients with NMI and *TSC2* mutations had similar incidences of AMLs and cysts. This paired comparison between NMI and *TSC1* patients showed no differences in renal cysts or angiomyolipoma. A similar comparison could not be performed for LAM due to the small number of age-matched pairs.

Family history

Ancestors: none of the NMI patients had family members in previous generations with confirmed TSC. Three NMI patients (14%) had family members with TSC skin features. Another patient reported two paternal aunts that had an illness that caused tumors to grow in many organs, her father was unaffected, but was not studied. None of the NMI patients had consanguineous parents.

Siblings: None of the NMI patients had siblings with confirmed TSC. Reproductive history was available for sixteen healthy mothers and five of them (31%) reported miscarriages prior to the delivery of the affected NMI patient.

Offspring: Five NMI women had a total of 14 children. Two (14%) of these children had confirmed TSC and two (14%) had possible TSC. Three mothers had unaffected children (2,3 and 2 respectively). One mother had 2 possibly affected children (skin features) and 2 healthy children; no genetic testing was performed. Another mother had one healthy child and 2 deceased with TSC: one due to cardiac rhabdomyomas, and one fetal loss at 7 months of gestational age; TSC was confirmed by autopsy in both cases; no genetic testing was performed. None of the male patients with NMI had children.

Comparison with previous NMI phenotypic characterizations

There is considerable similarity between the findings in this well-characterized set of NMI TSC patients, and those reported previously (Table 3). There were no significant differences between the frequency of any clinical feature in this NMI population and those reported by Au (Au et al. 2007), Sancak or Dabora, except for cognitive impairment that was lower in our NMI group (Dabora et al. 2001; Sancak et al. 2005).

Further characterization of NMI patients with LAM

Pulmonary LAM was present in 4/5 NMI women that were older than 16 years and had a high-resolution chest CT. These four women with NMI and LAM had never had seizures, had normal cognitive development, and all four had renal AMLs measuring more than 2 cm, including two with severe renal involvement: one had a nephrectomy at 16 years of age due to AML bleeding, and a 4.5 × 5.8 cm AML in the remaining kidney; the other had bilateral giant renal AML masses measuring 23 cm and 25.8 cm exerting mass effect on the liver and bowel. These four women were diagnosed with clinical TSC due to the presence of other major TSC criteria, as 3 of 4 had facial angiofibroma, and 3 of 4 had tubers (Table 4). Although the radiographic diagnosis of LAM was felt to be clear, none had respiratory symptoms, three had been pregnant (with 2, 3 and 4 pregnancies respectively), and three of them had had normal pulmonary function tests.

Discussion

We have described the clinical and genetic findings in a substantial cohort of TSC patients seen at one institution, with the objective of better defining the clinical characteristics of those TSC patients in whom no mutation is identified (NMI) after comprehensive testing for all common mutations by standard methods. Only index cases were included to avoid bias towards repeated sampling of the same mutation. We found no mutation in 11% (23/205) TSC patients, though note that 36 (17%) were excluded from consideration as they did not have TSC2 deletion analysis performed or had a sequencing variant of uncertain significance. This percentage is similar to previous findings (Dabora et al. 2001) (Sancak et al. 2005; Au et al. 2007). Large TSC2 deletions were found in 18/205 (9%) of our TSC population, representing 18/106 (17%) of the TSC2 cases, similar to previous findings (Dabora et al. 2001) (Kozlowski et al. 2007).

The clinical features in our NMI group were remarkably similar to those seen in previous studies except for cognitive impairment (Dabora et al. 2001; Sancak et al. 2005; Au et al. 2007). This could be due to different ascertainment methods or to the fact that patients with large *TSC2* deletions were excluded in our study. Overall NMI patients have milder brain, neurological and renal features than patients with *TSC2* mutations, but gender is a significant factor for renal AMLs: women with NMI and *TSC2* have a similar incidences of renal AML, whereas men with NMI have lower incidences compared to men with *TSC2*. In contrast, NMI patients have somewhat milder brain features than *TSC1* patients, but have more severe renal angiomyolipomas and LAM.

We observed a high frequency of LAM in the adult NMI women, four out of five (80%).

LAM incidence for the total sample (13/38 females ≥ 16 years, 42%) was similar to that seen in other series when high resolution chest CT was used for detection (Franz et al. 2001; Moss et al. 2001). LAM in TSC has a higher incidence in patients with *TSC2* than *TSC1* mutations and is almost always accompanied by renal AML (Franz et al. 2001). Somatic *TSC2* mutation combined with loss of the other *TSC2* allele ('LOH') has been demonstrated in AML cells and pulmonary LAM cells from patients with sporadic (non-TSC) LAM, supporting the model that sporadic LAM results from bi-allelic somatic *TSC2* mutation in cells that give rise to both pathologic lesions (Carsillo et al. 2000). In our study all NMI patients with LAM also had renal AML. All of the NMI patients with AMLs and LAM also had other diagnostic features of TSC, though they were relatively mildly affected, consistent with the general pattern among NMI patients.

There are at least three possible reasons for NMI in TSC patients: 1) mutation detection failure; 2) occurrence of a third TSC locus, currently unknown; and 3) mosaicism for mutations in *TSC1/TSC2* genes. It is certain that mutation detection failure accounts for some NMI patients, as small mutations that affect transcription or mRNA processing may occur in locations remote from coding exons (Mayer et al. 2000), and will not be detected by current molecular diagnostic methods. Even though these mutations account for a small fraction of all disease-causing mutations, they might constitute a significant percentage of the NMI group. Whole or multiple exon deletions in the *TSC1* gene have been shown to occur much less frequently than *TSC2* deletions (Kozlowski et al. 2007) and were not screened for in the analyzed cohort; they could also constitute a small fraction of the NMI group.

There is currently no substantial evidence for a third TSC gene locus beyond the occurrence of NMI TSC patients. The biochemical signaling pathway which the *TSC1/TSC2* protein complex regulates does not suggest that there are other genes that might cause an identical syndrome. Two other genes in the PI3K-mTOR signaling pathway cause related syndromes (*LKB1*, Peutz-Jeghers syndrome; *PTEN*, a variety of syndromes), but these are clinically quite distinct from TSC. Nonetheless, this remains a possibility.

Mosaicism is very likely to contribute to a fraction of NMI patients. Mosaicism is well documented in TSC, and can cause disease that ranges in severity from somewhat milder than average to classic and severe. Mosaicism has been seen in as many as 26% of index TSC patients who have large genomic deletions and rearrangements in *TSC2* (Sampson et al. 1997). One of us (DJK) has also recently confirmed that mosaicism is relatively common among patients with large genomic changes in *TSC1* and *TSC2*, being seen in 8 of 54 (15%) index patients. However, it appears to be much less common than this for the common small mutations in *TSC1* and *TSC2*, though the true incidence is uncertain due to mosaicism detection limitations (Verhoef et al. 1995; Kwiatkowska et al. 1999; Dabora et al. 2001; Sampson. 2003; Roberts et al. 2004).

In our NMI patients, there were none with affected parents whereas two adult NMI women transmitted the condition to their children, consistent with the possibility of germline mosaicism. In addition, the mild clinical features of the adult NMI women with LAM in this study is also consistent with germline mosaicism. This raises the possibility that a larger fraction of women diagnosed with LAM and AMLs have undetected TSC. However, this issue is typically carefully considered in LAM patients, and moreover there are no case reports of transmission of TSC to the children of a woman with LAM but not TSC, arguing against germline mosaicism with mild expression as a pathogenetic mechanism in LAM.

In summary, the clinical features of the NMI patients seen at our institution appears to be distinct from that of patients with either *TSC1* or *TSC2* mutations. These findings of a distinct TSC NMI phenotype is now consistent among 4 large series. Although mutation detection failure and mosaicism are each likely to account for a fraction of these patients, the frequency of each in NMI patients is currently unknown. Nonetheless, these consistent findings provide some guidance to clinicians caring for TSC patients who are NMI, mainly regarding active screening for renal and pulmonary involvement in spite of a mild neurological phenotype and a largely asymptomatic period during young adulthood. Hopefully future advances in genomic technology and medical application will lead to identification of causative mutations and clarify disease pathogenesis in NMI patients.

Acknowledgments

This work was supported by the Carol and James Herscot Center for Tuberous Sclerosis Complex, and NIH NINDS R01 NS31535. The authors are thankful to Elkan Halpern for his statistical expertise, and to Elisabeth Winterkorn and Kerry Conant for reviewing the manuscript.

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Table 1

	NMI (n = 23)	TSC2 (n = 94)	TSC1 (n = 40)	NMI v TSC2 P value	NMI v TSC1 P value
Age range					
Mean (st. deviation)	21.8 (15.5)	16.6 (12.0)	23.9 (15.6)	0.089	0.485
Female/Male	12/11	44/50	23/17	0.6443	0.6822
Neurological Features					
Seizures	13/23 56%	87/94 93%	34/40 85%	<.0001	0.0124
Infantile Spasms	4/23 17%	58/94 62%	5/40 13%	0.0001	0.5932
Cognitive impairment	4/23 17%	59/94 63%	15/40 38%	<.0001	0.0941
Brain Involvement					
Subependymal Nodules	16/23 70%	94/94 100%	34/40 85%	<.0001	0.1368
SGCT	3/23 13%	34/94 36%	10/40 25%	0.0325	0.259
Tubers	18/23 78%	92/94 98%	33/40 83%	0.0004	0.6801
Skin Involvement	21/23 91%	89/94 95%	40/40 100%	0.5408	0.0581
Age at renal imaging *					
Range	1.2–50.9	0.1–50.6	1.8 – 64.4		
Mean (st. deviation)	21.1 (14.8)	16.8 (12.0)	24.6 (14.6)	0.139	0.364
Proportion that had imaging	22/23 96%	93/94 (99%)	37/40 (93%)	0.2761	0.6213
Renal Involvement					
Cysts	4/21 19%	53/93 57%	12/37 32%	0.0017	0.0641
Angiomyolipomas	9/21 43%	64/93 69%	6/37 16%	0.0251	0.026
Proportion of women > 16 years who had chest CT	5/6 83%	19/27 70%	10/15 67%	0.519	0.445
Age at chest CT					
Age range	25 – 52	16 – 45	18 – 62		
Mean (st. deviation)	42.2 (10.9)	27.9 (10.7)	35.7 (13.6)	0.016	0.378

	NMI		TSC2		TSC1		NMI v TSC2		NMI v TSC1	
	(n = 23)		(n= 94)		(n= 40)		P value		P value	
LAM*** (F > 16y)	4/5	80%	8/18	44%	2/10	20%	0.1591		0.0253	

Table 2

Age matched comparison for renal features in each gender (Mc Nemar's)

	NMI/TSC2		NMI/TSC1	
	Male N= 12 pairs	Female N=10 pairs	Male N=9 pairs	Female N=8 pairs
Renal Involvement	P value	P value	P value	P value
Cysts	0.0039	0.0625	1.0	0.625
Angiomyolipomas	0.0156	0.625	1.0	0.125

Table 3

Comparison to previously published NMI samples (χ^2)

	NMI MGH	NMI Dabora	NMI Sancak	NMI Au	MGH/DaboraMGH/Sancak	MGH/Au
	(n = 23)	(n=38)	(n=27)		P value	P value
Age range						
Mean (st. deviation)	21.8 (15.5)	9.8	unknown	11.6 (9.1)	n/a	n/a
Female/Male	12/11		unknown	68/37	n/a	0.2586
Neurological Features						
Seizures	13/23 56%	25/37 68%	17/25 68%	56/94 60%	0.3881	0.4119
Infantile Spasms	4/23 17%			10/50 20%	n/a	n/a
Cognitive impairment	4/23 17%	12/22 54%	8/22 36%	27/70 39%	0.0092	0.1476
Brain Involvement						
Subependymal Nodules	16/23 70%	26/34 76%	18/22 82%	45/67 67%	0.5613	0.3391
SGCT	3/23 13%	2/37 5%	1/14 7%	9/69 13%	0.298	0.5752
Tubers	18/23 78%	19/24 79%	14/18 78%	49/69 71%	0.9805	0.9702
Renal Involvement						
Cysts	4/21 19%	6/34 18%	5/16 31%	20/67 30%	0.896	0.3914
Angiomyolipomas	9/21 43%	16/36 46%	9/16 56%	25/64 39%	0.9072	0.4194

Table 4

Major TSC features found in four female patients with pulmonary lymphangioleiomyomatosis and renal angiomyolipomas

	1	2	3	4
Facial angiofibroma	No	Yes	Yes	Yes
Forehead plaque	No	No	Yes	No
Periungual fibroma	No	No	Yes	Yes
Hypomelanotic macule	No	Yes	No	No
Shagreen patch	No	No	Yes	No
Retinal hamartoma	No	No	Yes	No
Subependymal nodules	No	Yes	No	No
Subependymal giant cell tumor	No	No	No	No
Tuber	Yes	Yes	Yes	No
Cardiac rhabdomyoma	No	No	No	Yes