Original article

Epileptic Disord 2014; 16 (4): 449-55

Mutational analysis of paediatric patients with tuberous sclerosis complex in Korea: genotype and epilepsy

Jin Sook Lee [1], Byung Chan Lim [1], Jong-Hee Chae [1], Yong Seung Hwang [1], Moon-Woo Seong [2], Sung Sup Park [2], Ki Joong Kim [1]

[1] Department of Pediatrics, Pediatric Clinical Neuroscience Center, Seoul National University Children's Hospital
[2] Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea

Received June 16, 2014; Accepted September 23, 2014

ABSTRACT – To date, only a few studies have reported that, in tuberous sclerosis, *TSC2* mutations are more frequently associated with infantile spasms and cognitive impairment compared to *TSC1* mutations. We analyzed the mutational spectrum of patients with tuberous sclerosis in Korea and attempted to explore the associations between genotype and seizure type/outcome. We performed mutational analyses on 70 unrelated patients with clinically confirmed tuberous sclerosis by using direct DNA sequencing and/or multiplex ligation-dependent probe amplification. The patients' medical records, including epilepsy type and outcome, were reviewed retrospectively. We identified pathogenic mutations in 55 patients (79%), 25 of which were novel. There were 12 *TSC1* mutations and 43 *TSC2* mutations. *TSC1* mutations included 8 frameshift and 4 nonsense mutations. *TSC2* mutations included 12 frameshift, 10 nonsense, 6 splicing, and 6 missense mutations, as well as 4 in-frame deletions and 5 large deletions. Fifty-eight patients had epilepsy (83%), including 19 patients with a history of infantile spasms. Compared to patients with *TSC1* mutations, individuals with *TSC2* mutations had a significantly higher frequency of epilepsy ($p<0.05$) and tended to have a higher frequency of infantile spasms (37% vs 17%; $p<0.3$). Most of the patients with *TSC2* mutations who developed infantile spasms exhibited subsequent epilepsy (13/14; 93%). However, the presence/absence of infantile spasms did not influence seizure remission or cognitive outcome.

Key words: tuberous sclerosis complex, *TSC1*, *TSC2*, genotype, seizure type, outcome

Correspondence:
Ki Joong Kim
Department of Pediatrics,
Pediatric Clinical Neuroscience Center,
Seoul National University Children's Hospital,
Seoul National University College of Medicine,
101 Daehakro Jongno-gu,
Seoul, 110-744 Korea
<pednr@plaza.snu.ac.kr>

doi:10.1684/epd.2014.0712

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that involves multiple organs and tissues. It is caused by a mutation in either *TSC1* or *TSC2*, which are tumour suppressor genes. TSC exhibits a wide spectrum of clinical manifestations, which may be associated with genetic heterogeneity and incomplete penetrance (Baraitser and Patton, 1985; Osborne *et al.*, 2000; Dabora *et al.*, 2001; Sancak *et al.*, 2005; Lyczkowski *et al.*, 2007). The neurological manifestations of the disease include epilepsy, intellectual disability, focal neurological deficits, and hydrocephalus, which result from specific brain lesions such as tubers or subependymal giant cell astrocytomas (SEGAs). Among these, epilepsy is the most common and highly morbid feature. The pathophysiological mechanism of epilepsy in TSC remains unknown, although it is reported to correlate with tubers (Doherty *et al.*, 2005; Feliciano *et al.*, 2013; Meng *et al.*, 2013).

Hamartin and tuberin, which are encoded by the *TSC1* and *TSC2* genes, respectively, form a protein complex that activates the GTPase activity of Rheb, thus inhibiting the mammalian target of rapamycin (mTOR) pathway. The mammalian target of rapamycin complex 1 is a serine-threonine kinase that plays a major role in cell growth signalling. Many studies of the mTOR pathway related to tumourigenesis have been performed (Jozwiak *et al.*, 2008; Crino, 2013; Feliciano *et al.*, 2013). However, there are only a few reports about the association between epilepsy and the mTOR pathway (McDaniel and Wong, 2011; Meng *et al.*, 2013), although in the brain this pathway has been shown recently to function in synaptic plasticity and axonal or dendritic growth (Hoeffer and Klann, 2010; Crino, 2011).

Some large cohort studies showed that patients with *TSC2* mutations tended to have a more severe phenotype compared to patients with *TSC1* mutations or no identified mutations (Dabora *et al.*, 2001; Sancak *et al.*, 2005; Au *et al.*, 2007; Jansen *et al.*, 2008; van Eeghen *et al.*, 2012). Furthermore, it was suggested recently that variable neurocognitive features are associated with different locations as well as types of *TSC* germline mutations (van Eeghen *et al.*, 2012). However, there are only a few studies showing that *TSC2* mutations are associated more frequently with infantile spasms and cognitive impairment, compared to *TSC1* mutations (Chu-Shore *et al.*, 2010; van Eeghen *et al.*, 2013; Vignoli *et al.*, 2013).

We investigated the distribution and spectrum of *TSC* mutations in the Korean population and attempted to correlate seizure type and outcome with genotype. It would be helpful to uncover the pathophysiological mechanism of epilepsy in TSC.

Materials and Methods

Patient and clinical data

Seventy unrelated patients diagnosed with TSC at the Seoul National University Children's Hospital (SNUCH) were included in the present study (Roach and Sparagana, 2004). Genetic testing was performed using direct sequencing and/or multiplex ligation-dependent probe amplification. The cohort included 42 newly enrolled patients with TSC and 15 patients from the SNUCH TSC repository in whom no molecular defects had been identified based only on denaturing high-performance liquid chromatography analysis. Thirteen patients who were reported to have *TSC* mutations in a previous study (Choi *et al.*, 2006) were also confirmed as having the molecular defects and were included in the clinical analysis.

The patients' medical records were reviewed retrospectively. Epilepsy was defined as at least two unprovoked clinical seizures. Epilepsy types were classified based on clinical semiology and interictal/ictal electroencephalography (EEG):

– infantile spasms;
– focal epilepsy;
– generalized epilepsy;
– undetermined epilepsy.

Seizure outcomes were evaluated for at least one year, and the last visit to the clinic in which seizure status was documented was used as the endpoint of the follow-up; a seizure-free status (one-year seizure remission) was established when patients had no seizures. Cases in which the duration of the follow-up was less than one year were excluded from the analysis of seizure outcomes. Cognitive impairment was also evaluated using a neuropsychological test (Wechsler Intelligence Scale for Children, KEDI-WISC), when possible. Otherwise, developmental milestones were checked by the paediatric neurologists. Brain magnetic resonance imaging (MRI) was performed at least once for all the enrolled patients, with the exception of three patients in whom brain computed tomography (CT) had been performed in a previous study. This study was approved by the institutional review boards of SNUCH.

Mutational analysis

Blood samples were obtained from enrolled patients who provided informed consent. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA) or a Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's instructions. Direct sequencing of the entire coding exons and flanking intronic sequences of the *TSC1* and *TSC2* genes was performed using primer pairs that were designed by the authors, which are available upon request, or using Primer 3 (http://frodo.wi.mit.edu/) and the Refseq of *TSC1* (NM_000368.4) and *TSC2* (NM_000548.3). Polymerase chain reaction amplification was performed on a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA) and cycle sequencing was performed on an ABI Prism 3730xl DNA Analyzer using the BigDye Terminator Sequencing Ready Reaction Kit (Applied Biosystems) or an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence variations were analyzed via comparison with the wild-type sequence or using the Seqscape v2.5 software. The mutation nomenclature followed the recommendations of the Human Genome Variation Society. A nucleotide change was considered pathogenic when it:

– was confirmed by comparing with the *TSC1*/*TSC2* Leiden Open Variation Database (http://chromium.liacs.nl/LOVD2/TSC);

– resulted in protein truncation;

– was not observed in either parent and paternity was confirmed in the cases where parental DNA was collected and tested for the presence of the identified variants.

The significance of novel missense variations was evaluated using the following methods:

– allele frequencies were screened in 100 ethnically-matched normal subjects;

– segregation patterns were analyzed among the family members that were available.

The mutational properties of intronic variations were predicted using automated splicing mutation analysis. All candidate variants were searched in the *TSC1*/*TSC2* Leiden Open Variation Database to confirm their novelty (http://chromium.liacs.nl/LOVD2/TSC).

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed using the SALSA MLPA kit: (1) P124-B1 and P337-A2 or (2) P124-C1 and P046-C1 (MRC Holland, Amsterdam, the Netherlands), according to the manufacturer's instructions. The MLPA samples consisted of 50-100 ng of genomic DNA. Ligation and amplification were performed using a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). All amplified fragments were separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The area under the peak for each amplified fragment was measured and normalized to the peak areas of normal control individuals using the GeneMarker software, version 1.6 (SoftGenetics, State College, PA, USA). The reference range was set at 0.75-1.3.

Variants in the *TSC1* or *TSC2* gene were defined as unclassified when we were not able to establish whether they were pathogenic or not:

(1) Novel missense or splicing changes with uncertain pathogenicity;

(2) Synonymous substitutions;

(3) Single-nucleotide substitutions that had been reported before but carried uncertain significance.

Mutations were classified as protein-truncating (PT; nonsense, frameshift, and splicing mutations, as well as large deletions of at least one exon) and non-truncating (NT; missense mutations and small in-frame deletions and insertions) mutations. Moreover, they were classified according to which functional domains of the *TSC1* and *TSC2* gene products were affected; the tuberin-interaction domain (TID) in the *TSC1* gene, and the hamartin-interaction domain (HID) and the GTPase-activating protein (GAP) domain in the *TSC2* gene. We also investigated whether the locations of the mutation in the *TSC2* gene affected the phenotype, based on the following regions: proximal (E1-E22, including the HID), central (E23-E33), and distal (E34-E41, including the GAP domain) (van Eeghen *et al.*, 2012).

Statistical analysis

Statistical analyses were performed using SPSS Version 21 (SPSS, Inc., Chicago, IL, USA). We used the Kruskal-Wallis or Mann-Whitney *U* test for age variables and the chi-squared or Fisher's exact test for categorical variables. $p<0.05$ was considered significant.

Results

Patient characteristics

Table 1 lists the characteristics of patients and their clinical features. There were 36 females (51%) and 34 males (49%). Seventeen patients (24%) had a positive family history. The mean age at presentation and diagnosis was 20 and 33 months, respectively (range for both: 0-168 months), and was similar regardless of genotype. The mean duration of the follow-up was 108 months (range: 3-228 months). Fifty-eight patients developed epilepsy (83%) and 47 patients presented seizures (67%). With the exception of two patients with an incomplete history, the mean age at seizure onset was 27 months (range: 1-168 months). Nineteen patients had a history of infantile spasms (27%), more than half of whom had infantile spasms followed by seizures of other types, whereas four patients had spasms only and three patients had focal epilepsy that preceded spasms. Thirty-five patients had focal epilepsy without a history of infantile spasms. Two

Table 1. Patient characteristics and clinical features according to TSC genotype.

	Total (*n*=70)	TSC1 (*n*=12)	TSC2 (*n*=43)	NMI (*n*=9)	*p-value*
Sex (Male:Female)	34:36	6:6	23:20	3:6	0.65
Age at presentation (months)	19.6 (0-168)	28.4 (0-132)	16.6 (0-168)	23.9 (0-108)	0.58
Age at diagnosis (months)	33.0 (0-168)	43.9 (0-135)	28.1 (0-168)	52.0 (0-144)	0.50
Family history of TSC	17 (24%)	3 (25%)	8 (19%)	3 (33%)	0.60
Epilepsy	58 (83%)	7 (58%)	37 (86%)	9 (100%)	**0.03**
Presenting with seizures	47 (68%)	5 (42%)	29 (69%)	8 (89%)	0.09
Cognitive impairment	33 (47%)	2 (17%)	20 (47%)	7 (78%)	**0.02**
Brain tubers	62 (89%)	7 (58%)	41 (95%)	8 (89%)	**0.00**
SENs	61 (87%)	11 (92%)	38 (91%)	6 (67%)	0.13
SEGAs	16 (23%)	2 (17%)	12 (28%)	2 (22%)	0.91
Renal AML lesions	27 (39%)	0 (0%)	20 (47%)	6 (67%)	**0.00**
Cardia rhabdomyoma	37 (53%)	6 (50%)	24 (56%)	3 (33%)	0.46
Skin lesions	56/68 (82%)	8/11 (73%)	35/42 (83%)	9/9 (100%)	0.30
Retinal hamartoma	20/58 (34%)	0/9 (0%)	16/38 (42%)	3/6 (50%)	**0.03**

Age is presented as mean (range). TSC: tuberous sclerosis complex; NMI: no mutation identified; SENs: subependymal nodoules; SEGA: subependymal giant cell astrocytomas; AML: angiomyolipoma.
This table does not include patients with unclassified variants.

patients had generalized epilepsy, including one with atypical absence seizures and one with myoclonic seizures. The mean duration of the follow-up of seizure outcome in epileptic patients was 118 months (range: 3-228 months). Among the 51 patients with at least one year of follow-up, 35 (69%) had seizure remission regardless of seizure type. Thirteen of these patients (37%) received no medication. Cognitive impairment was present in 33 patients (47%) and was significantly associated with epilepsy (p=0.002; data not shown).

Mutation analysis

As described above, mutational analysis was performed for 70 TSC patients. Pathogenic mutations were identified in 55 patients (79%), including 12 *TSC1* mutations (22%) and 43 *TSC2* mutations (78%). No mutation was identified in nine patients. Six patients had unclassified variants.

TSC1 mutations included 8 frameshift (67%) and 4 nonsense (33%) mutations, with no missense or splicing mutations or large rearrangements. These included 3 mutations that affected the TID. Eight of the 12 *TSC1* mutations were novel mutations and 4 were known to be pathogenic. There was one *de novo* mutation from the four cases in which genetic tests of parental DNA were performed. In 3 patients, the fathers had the same frameshift mutation that was carried by the patients but did not have the clinical symptoms of TSC, although work-up for TSC was not performed for the fathers.

TSC2 mutations included 12 frameshift (28%), 10 nonsense (23%), 6 missense (14%), and 6 splicing mutations (14%), as well as 4 in-frame deletions (9%) and 5 large deletions (12%). They included 8 mutations that affected the HID, 3 mutations that inactivated the GAP domain, and 2 whole deletions that affected both domains. Seventeen of all *TSC2* mutations were novel mutations and 26 were known to be pathogenic. There were 15 *de novo* mutations from 18 samples for which parental DNA was tested. All 13 central *TSC2* mutations and all 8 HID mutations were protein-truncating, whereas all the non-truncating mutations were located in the distal regions, including the GAP domain, and in proximal regions (with the exception of the HID) of *TSC2*.

Genotype-phenotype association

Compared to individuals with *TSC1* mutations, patients with *TSC2* mutations had a significantly higher frequency of epilepsy ($p<0.05$). They were more likely to present with seizures, although this result did not reach significance because of the limited sam-

Table 2. Epilepsy and cognitive outcome in TSC patients with or without infantile spasms.

	TSC1 (*n*=12)	**TSC2 (*n*=43)**
Epilepsy with infantile spasms	2 (17%)	16 (37%)
spasms remission	na	12/14 (86%)
subsequent epilepsy	na	13/14 (93%)
subsequent seizure remission	na	9/13 (69%)
cognitive impairment	na	8/14 (57%)
Epilepsy without infantile spasms	5 (42%)	21 (49%)
focal epilepsy	4	19
generalized epilepsy	1	0
undetermined	0	2
seizure remission	3/5 (60%)	15/19 (79%)
cognitive impairment	1/5 (20%)	11/21 (52%)

na: not available. No results reached statistical significance.

ple size (p=0.10). Patients with *TSC2* mutations were more likely to have cognitive impairment compared to those with *TSC1* mutations (p<0.1). Patients with a history of infantile spasms included two individuals with *TSC1* mutations and 16 with *TSC2* mutations (17% vs 37%; p<0.3). Responses to vigabatrin, which was administered to manage infantile spasms, were very good, with complete remission in 86% of patients with *TSC2* mutations, although the two patients who had *TSC1* mutations were lost to follow-up and could not be evaluated. Ninety-three percent of patients with *TSC2* mutations who developed infantile spasms had subsequent epilepsy, and most of these cases were focal epilepsy. Moreover, cognitive impairment was observed in 57% of these patients. Among individuals without a history of infantile spasms, patients with *TSC2* mutations exhibited a satisfactory response to antiepileptic drugs, however, they showed poor cognitive outcome compared to those with *TSC1* mutations (p=0.33, *table 2*). Among patients with *TSC2* mutations, the presence/absence of infantile spasms did not influence seizure remission or cognitive outcome. In addition, we sorted the *TSC2* mutation group according to the location and type of mutation and compared their epilepsy type and outcome (data not shown). The presence of truncating mutations and the location of mutations in the *TSC2* group did not correlate with seizure type or outcome.

We also investigated the association of TSC genotypes with other clinical features. Compared to individuals with *TSC1* mutations, patients with *TSC2* mutations had a significantly higher frequency of brain tubers, renal angiomyolipomas, and retinal hamartomas (p=0.00, 0.00, and 0.02, respectively).

Discussion

The mutation detection rate obtained in the present study was 79%, which is similar to that reported by other studies (Dabora *et al.*, 2001; Sancak *et al.*, 2005). *TSC1* missense mutations are very rare, and we found no definite *TSC1* missense mutations. The distribution of other mutations is similar to that reported by previous studies, including the proportion of large deletions in the *TSC2* gene (Dabora *et al.*, 2001; Sancak *et al.*, 2005). These findings, which are different from those of other studies performed in Korea, might be explained by a more comprehensive method of mutational analysis and the relatively large population studied (Choi *et al.*, 2006; Jang *et al.*, 2012). Notably, this study showed a relatively high proportion of frameshift mutations in the *TSC1* gene. Large deletions in the *TSC2* gene were detected in five patients (12%), one of whom had a whole *TSC2* deletion with polycystic kidney disease, which is a contiguous gene syndrome. There was a recurrent *TSC2* mutation in three patients, which is a known small in-frame deletion (c.5238_5255del) (Niida *et al.*, 2013). A higher proportion of familial cases, in whom TSC was diagnosed in the family clinically and/or genetically, was observed in the *TSC1* mutation group compared to the *TSC2* mutation group, although genetic tests were not performed in all the parental samples (Dabora *et al.*, 2001; Sancak *et al.*, 2005). Interestingly, three patients had asymptomatic fathers with the same mutation as their own frameshift mutation in the *TSC1* gene. Unfortunately, we did not perform a work-up for TSC in their parents. The possibility of incomplete penetrance should always be considered in TSC and it seems to be detected more frequently in patients with *TSC1* mutations (Osborne *et al.*, 2000; Baraitser and Patton, 1985). The mother of one female patient with a frameshift mutation in the *TSC2* gene was diagnosed with definite TSC but did not carry the mutation. The patient's mother had facial angiofibromas and epilepsy during infancy with cortical tubers, and might have had somatic mosaicism, including germline mutation. Further studies, using techniques such as real-time qPCR, are needed to investigate this case. These findings suggest that the genotypic and phenotypic spectrum can be more variable than expected, therefore, the genetic analysis of patients and their parents/family members should be interpreted carefully taking into consideration the possibility of mosaicism and non- or incomplete penetrance.

Epilepsy is the most common neurological symptom and is the primary cause of medical attention in TSC patients. In the present study, epilepsy was the most common symptom (83%) and presentation (67%). Infantile spasms are the most morbid type of seizure, are related to poor cognitive outcome, and occur in about a third of patients with TSC (Chu-Shore *et al.*, 2010). This study showed that infantile spasms occurred in 27% of patients with TSC and represented 33% of the total seizures, demonstrating that this was a common presenting seizure type. We explored the association between epilepsy type, outcome, and the genotype of TSC. Patients with *TSC2* mutations were more likely to have epilepsy and present with seizures, notably infantile spasms, compared to those with *TSC1* mutations (Dabora *et al.*, 2001; Jansen *et al.*, 2008; Chu-Shore *et al.*, 2010; van Eeghen *et al.*, 2013; Vignoli *et al.*, 2013). Most of the individuals who had infantile spasms exhibited complete remission of spasms with good response to vigabatrin, as expected, although most of them had subsequent epilepsy. Conversely, the efficacy of vigabatrin for focal epilepsy in TSC patients was lower (55.6% vs 80%) than its efficacy for infantile spasms, although still relatively good (Yum *et al.*, 2013). Unfortunately, our study included only two patients with *TSC1* mutations who developed infantile spasms, who were both lost to follow-up. Thus, we were unable to perform statistical comparisons based on genotype. Among the individuals who did not have a history of infantile spasms, even patients with *TSC2* mutations exhibited a satisfactory response to antiepileptic drugs, which was inconsistent with previous findings (Chu-Shore *et al.*, 2010; Vignoli *et al.*, 2013). However, these patients showed a poor cognitive outcome compared to those with *TSC1* mutations. In addition, this study showed that the presence of infantile spasms did not influence seizure remission or cognitive outcome, although it was limited to patients with *TSC2* mutations. These findings might support the hypothesis that, in TSC patients, good control of infantile spasms with antiepileptic treatment (such as vigabatrin) leads to subsequent seizure remission and cognitive functioning comparable to that of patients without infantile spasms, unlike that observed in other patients with symptomatic infantile spasms (Fukushima *et al.*, 2001; Bombardieri *et al.*, 2010). We also suggest that *TSC2* mutation itself, and not the presence of infantile spasms, might have a greater influence on seizure outcome (Vignoli *et al.*, 2013). In addition, cognitive function might well be explained by germline mutations (Feliciano *et al.*, 2013). The seizure-free rate was relatively high in this study (69%), although about half of the patients were taking more than two antiepileptic drugs and the duration of the follow-up was limited (Chu-Shore *et al.*, 2010; Vignoli *et al.*, 2013). These findings might mean that TSC epileptic patients are more likely to be controlled in childhood, regardless of seizure type, by adding multiple antiepileptic drugs, however, seizures can be aggravated with time because of pathological lesions.

This is the largest cohort-based TSC mutational analysis performed in Korea. The importance of genetic testing is such that it is included in the new 2012 diagnostic criteria for TSC (Roach and Sparagana, 2004; Northrup and Krueger, 2013). The 25 novel mutations identified in this study expand the genetic spectrum of TSC. As expected, patients with *TSC2* mutations tended to have a higher frequency of epilepsy, notably infantile spasms, and cognitive impairment, compared to those with *TSC1* mutations. However, the presence of infantile spasms did not affect subsequent seizure remission or cognitive outcome among patients with *TSC2* mutations.

Acknowledgements and disclosures.

This study was supported by a grant from the Korea Healthcare Technology R&D project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (Grant No. A120099).

This study was approved by the local institution review board.

The authors declare no conflicts of interest with respect to the research, authorship, or publication of this article.

References

Au KS, Williams AT, Roach ES, *et al*. Genotype/phenotype correlation in 325 individuals referred for a diagnosis of tuberous sclerosis complex in the United States. *Genet Med* 2007; 9: 88-100.

Baraitser M, Patton MA. Reduced penetrance in tuberous sclerosis. *J Med Genet* 1985; 22: 29-31.

Bombardieri R, Pinci M, Moavero R, Cerminara C, Curatolo P. Early control of seizures improves long-term outcome in children with tuberous sclerosis complex. *Eur J Paediatr Neurol* 2010; 14: 146-9.

Choi JE, Chae JH, Hwang YS, Kim KJ. Mutational analysis of TSC1 and TSC2 in Korean patients with tuberous sclerosis complex. *Brain Dev* 2006; 28: 440-6.

Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010; 51: 1236-41.

Crino PB. mTOR: A pathogenic signaling pathway in developmental brain malformations. *Trends Mol Med* 2011; 17: 734-42.

Crino PB. Evolving neurobiology of tuberous sclerosis complex. *Acta Neuropathol* 2013; 125: 317-32.

Dabora SL, Jozwiak S, Franz DN, *et al*. Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of TSC2, compared with TSC1, disease in multiple organs. *Am J Hum Genet* 2001; 68: 64-80.

Doherty C, Goh S, Young Poussaint T, Erdag N, Thiele EA. Prognostic significance of tuber count and location in tuberous sclerosis complex. *J Child Neurol* 2005; 20: 837-41.

Feliciano DM, Lin TV, Hartman NW, *et al*. A circuitry and biochemical basis for tuberous sclerosis symptoms: From epilepsy to neurocognitive deficits. *Int J Dev Neurosci* 2013; 31: 667-78.

Fukushima K, Inoue Y, Fujiwara T, Yagi K. Long-term follow-up study of West syndrome associated with tuberous sclerosis. *Brain Dev* 2001; 23: 698-704.

Hoeffer CA, Klann E. mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends Neurosci* 2010; 33: 67-75.

Jang MA, Hong SB, Lee JH, *et al*. Identification of TSC1 and TSC2 mutations in Korean patients with tuberous sclerosis complex. *Pediatr Neurol* 2012; 46: 222-4.

Jansen FE, Braams O, Vincken KL, *et al*. Overlapping neurologic and cognitive phenotypes in patients with TSC1 or TSC2 mutations. *Neurology* 2008; 70: 908-15.

Jozwiak J, Jozwiak S, Wlodarski P. Possible mechanisms of disease development in tuberous sclerosis. *Lancet Oncol* 2008; 9: 73-9.

Lyczkowski DA, Conant KD, Pulsifer MB, *et al*. Intrafamilial phenotypic variability in tuberous sclerosis complex. *J Child Neurol* 2007; 22: 1348-55.

McDaniel SS, Wong M. Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis. *Neurosci Lett* 2011; 497: 231-9.

Meng XF, Yu JT, Song JH, Chi S, Tan L. Role of the mTOR signaling pathway in epilepsy. *J Neurol Sci* 2013; 332: 4-15.

Niida Y, Wakisaka A, Tsuji T, *et al*. Mutational analysis of TSC1 and TSC2 in Japanese patients with tuberous sclerosis complex revealed higher incidence of TSC1 patients than previously reported. *J Hum Genet* 2013; 58: 216-25.

Northrup H, Krueger DA. Tuberous sclerosis complex diagnostic criteria update: Recommendations of the 2012 international tuberous sclerosis complex consensus conference. *Pediatr Neurol* 2013; 49: 243-54.

Osborne JP, Jones AC, Burley MW, *et al*. Non-penetrance in tuberous sclerosis. *Lancet* 2000; 355: 1698.

Roach ES, Sparagana SP. Diagnosis of tuberous sclerosis complex. *J Child Neurol* 2004; 19: 643-9.

Sancak O, Nellist M, Goedbloed M, *et al*. Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: Genotype-phenotype correlations and comparison of diagnostic DNA techniques in Tuberous Sclerosis Complex. *Eur J Hum Genet* 2005; 13: 731-41.

van Eeghen AM, Black ME, Pulsifer MB, Kwiatkowski DJ, Thiele EA. Genotype and cognitive phenotype of patients with tuberous sclerosis complex. *Eur J Hum Genet* 2012; 20: 510-5.

van Eeghen AM, Nellist M, van Eeghen EE, Thiele EA. Central TSC2 missense mutations are associated with a reduced risk of infantile spasms. *Epilepsy Res* 2013; 103: 83-7.

Vignoli A, La Briola F, Turner K, *et al*. Epilepsy in TSC: Certain etiology does not mean certain prognosis. *Epilepsia* 2013; 54: 2134-42.

Yum MS, Lee EH, Ko TS. Vigabatrin and mental retardation in tuberous sclerosis: Infantile spasms versus focal seizures. *J Child Neurol* 2013; 28: 308-13.

TEST YOURSELF

(1) Which genotype is more closely associated with epilepsy, notably infantile spasms, in patients with tuberous sclerosis complex?

(2) Familial TSC cases appear to be observed more frequently in the *TSC2* mutation group compared to the *TSC1* mutation group. Is this true?

Note: Reading the manuscript provides an answer to all questions. You can check for the correct answer by visiting the Educational Centre section of www.epilepticdisorders.com