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

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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.

Keywords: tuberous sclerosis complex, TSC1, TSC2, genotype, seizure type, outcome
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 germ line 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 (Genta 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.

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

Age is presented as mean (range). TSC: tuberous sclerosis complex; NMI: no mutation identified; SENs: subependymal nodules; SEGAs: 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.

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

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.

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References


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**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](http://www.epilepticdisorders.com)*