Mutational spectrum of the TSC1 gene in a cohort of 225 tuberous sclerosis complex patients: no evidence for genotype-phenotype correlation

Marjon van Slegtenhorst, Senno Verhoef, Anita Tempelaars, Lida Bakker, Qi Wang, Marja Wessels, Remco Bakker, Mark Nellist, Dick Lindhout, Dicky Halley, Ans van den Ouweland

Abstract
Tuberous sclerosis complex is an inherited tumour suppressor syndrome, caused by a mutation in either the TSC1 or TSC2 gene. The disease is characterised by a broad phenotypic spectrum that can include seizures, mental retardation, renal dysfunction, and dermatological abnormalities. The TSC1 gene was recently identified and has 23 exons, spanning 45 kb of genomic DNA, and encoding an 8.6 kb mRNA. After screening all 21 coding exons in our collection of 225 unrelated patients, only 29 small mutations were detected, suggesting that TSC1 mutations are under-represented among TSC patients. Almost all TSC1 mutations were small changes leading to a truncated protein, except for a splice site mutation and two in frame deletions in exon 7 and exon 15. No clear difference was observed in the clinical phenotype of patients with an in frame deletion or a frameshift or nonsense mutation. We found the disease causing mutation in 13% of our unrelated set of TSC patients, with more than half of the mutations clustered in exons 15 and 17, and no obvious under-representation of mutations among sporadic cases. In conclusion, we find no support for a genotype-phenotype correlation for the group of TSC1 patients compared to the overall population of TSC patients.

Keywords: tuberous sclerosis complex; mutations; genotype-phenotype correlation

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder characterised by the growth of hamartomas in many tissues and organs, including brain, skin, heart, and kidney. Common neurological manifestations including seizures and mental retardation have their onset during early childhood, while cysts and angiomylipomas in the kidney mostly become apparent during adult life. Considerable clinical variation is observed between as well as within families. At least 60% of TSC patients represent sporadic cases and whether there is a reduced risk of mental retardation in TSC1 related disease. The goal of this study was to construct the mutational spectrum of the TSC1 gene in our collection of TSC patient samples by Southern blot and SSCP analysis. This would enable us to determine whether there is a significant difference in the detection rate between familial and sporadic cases and whether there is a genotype-phenotype correlation for the TSC1 group compared to the overall population of TSC patients.

Patients and methods

PATIENT SELECTION AND DNA ISOLATION
In this study, 225 unrelated patients with tuberous sclerosis complex, diagnosed according to the criteria of Gomez, were included. Eighty-two patients represented familial cases (36%) with at least one first degree relative. Three large families showed linkage to 9q34. One hundred and forty-three patients were designated sporadic cases (64%), in the absence of an apparent family history of TSC. Patients with a TSC2 mutation were excluded from the 225 cases. DNA was isolated from peripheral blood cells according to standard
Paternity tests were performed using the Profile kit from Perkin Elmer.

**SOUTHERN BLOTTING ANALYSIS**

Genomic DNA (6 µg) of 200 unrelated patients was digested with four different restriction enzymes (EcoRI, HindIII, PstI, and TaqI) and run on a 0.7% agarose gel. Southern blotting and hybridisation were performed using standard methods. Three cDNA clones were tested on the blots: a 5'RACE clone which had been amplified from a fetal brain cDNA pool (bp 24-1696) (Clontech), an RT-PCR product (bp 1616-3684) generated from fibroblast RNA, and a fetal brain cDNA clone (bp 4100-8600). The three probes cover the coding sequence as well as the 5' and 3' untranslated region of the TSC1 gene.

**SSCP ANALYSIS AND DNA SEQUENCING**

Sequences of primers used for amplification of the 21 coding exons of the TSC1 gene are provided at http://expmed.bwh.harvard.edu/projects/tsc/. For exon 22, a new intronic forward primer was designed (5'-atactaccagctactttcata-3'). SSCP analysis was performed according to Orita et al and 2 µl of the PCR product was applied to the Pharmacia GenePhor Electrophoresis system. Gels were run for 2.5 hours at 5°C and 18°C. Running conditions for two gels were 600 V, 50 mA, and 10 W. Subsequently, bands were visualised using a DNA silver staining kit (Pharmacia) in a Hoefer automated gel stainer. Variant patterns were further characterised by direct sequence analysis of the PCR products on an automated DNA sequencer (ABI 377) using the cycle sequencing dye primer kit (Perkin Elmer).

**ASO HYBRIDISATION**

Oligonucleotides for ASO hybridisation were designed for the mutated and normal sequence. ASO hybridisations were performed at 37°C for 30 minutes. Filters were washed to 0.3 x SSC for 10 minutes at 37°C.

**Results**

**SCREENING FOR LARGE ABNORMALITIES**

No large insertions or deletions were identified in the TSC1 gene by Southern analysis in 200 patients. Only in one case (T2965) was an aberrant restriction pattern in a TaqI digest detected (fig 1A), but no consistent change was seen with other enzymes. Comparing the genomic sequence of the TSC1 gene with the size of the extra fragment, we were able to locate the lost TaqI site in exon 15. Sequencing exon 15 of the TSC1 gene of this patient showed a C to T substitution at bp position 1719 (fig 1B), resulting in the nonsense mutation R500X. The presence of this mutation was confirmed by allele specific oligonucleotide (ASO) hybridisation (fig 1C).

**SCREENING FOR SMALLER MUTATIONS**

Systematic SSCP analysis was undertaken to screen the 21 coding exons in the TSC1 gene for small mutations. In total, 25 different mutations were found in 29 subjects on an set of 225 unrelated patients (table 1). All types of mutations resulting in a truncated protein have been observed: small deletions/insertions,

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**Table 1** Mutations identified in the TSC1 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Patient code</th>
<th>Mutation (nt substitution)</th>
<th>Familial/ sporadic</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>T2545</td>
<td>367delT</td>
<td>Familial</td>
</tr>
<tr>
<td>5</td>
<td>T1214</td>
<td>W101X (529G→A)</td>
<td>Familial</td>
</tr>
<tr>
<td>7</td>
<td>T1817</td>
<td>432-1G→A</td>
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</tr>
<tr>
<td>7</td>
<td>T1298</td>
<td>814delACCT</td>
<td>Familial</td>
</tr>
<tr>
<td>8</td>
<td>T4715</td>
<td>94kitaA</td>
<td>Familial</td>
</tr>
<tr>
<td>8</td>
<td>T8129</td>
<td>958delG</td>
<td>Familial</td>
</tr>
<tr>
<td>9</td>
<td>T7806</td>
<td>R249X (966A→T)</td>
<td>Familial</td>
</tr>
<tr>
<td>9</td>
<td>T1207</td>
<td>1210insT</td>
<td>Familial</td>
</tr>
<tr>
<td>10</td>
<td>T3945</td>
<td>1240delA</td>
<td>Familial</td>
</tr>
<tr>
<td>11</td>
<td>T9809</td>
<td>Y312X (1575C→A)</td>
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</tr>
<tr>
<td>12</td>
<td>T10301</td>
<td>143edIC</td>
<td>Familial</td>
</tr>
<tr>
<td>13</td>
<td>T1515‡</td>
<td>1499edCT</td>
<td>Familial</td>
</tr>
<tr>
<td>15</td>
<td>T2965</td>
<td>R500X (1719C→T)</td>
<td>Familial</td>
</tr>
<tr>
<td>15</td>
<td>T9886</td>
<td>R509X (1746C→T)</td>
<td>Familial</td>
</tr>
<tr>
<td>15</td>
<td>T3922</td>
<td>1892del123</td>
<td>Familial†</td>
</tr>
<tr>
<td>15</td>
<td>T2067</td>
<td>1929delAG*</td>
<td>Familial</td>
</tr>
<tr>
<td>15</td>
<td>T5913</td>
<td>1978del9</td>
<td>Familial†</td>
</tr>
<tr>
<td>15</td>
<td>T2636</td>
<td>2007delT*</td>
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</tr>
<tr>
<td>15</td>
<td>T4124</td>
<td>2105delAAG*</td>
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<td>17</td>
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<td>Familial†</td>
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<td>Familial†</td>
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<td>Familial</td>
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<tr>
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<td>T7659</td>
<td>2318ins28</td>
<td>Familial</td>
</tr>
<tr>
<td>17</td>
<td>T4068</td>
<td>3238delGCT</td>
<td>Familial</td>
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<tr>
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<td>T2077‡</td>
<td>R786X (2577C→T)</td>
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<td>T9886</td>
<td>R509X (1746C→T)</td>
<td>Familial</td>
</tr>
<tr>
<td>20</td>
<td>T3922</td>
<td>1892del123</td>
<td>Familial†</td>
</tr>
<tr>
<td>20</td>
<td>T1295</td>
<td>1892del123</td>
<td>Familial</td>
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<tr>
<td>20</td>
<td>T10816</td>
<td>1892del123</td>
<td>Familial†</td>
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<tr>
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<td>2318ins28</td>
<td>Familial</td>
</tr>
<tr>
<td>20</td>
<td>T4068</td>
<td>3238delGCT</td>
<td>Familial</td>
</tr>
</tbody>
</table>

nt = nucleotide; *mutation reported before; †parents tested negative for the mutation; ‡family linked to chromosome 9; §recurrent mutation, also identified by other groups.
nonsense mutations, and splice site mutations. In addition, eight different missense changes (table 2) were seen in nine unrelated patients.

Fourteen of the 29 mutations were small deletions, ranging from 1 to 23 bp. Three of these mutations have been reported previously. In two patients, we detected in frame deletions of 3 and 9 bp respectively. In family T1298, a 3 bp deletion in exon 7 segregated with the disease phenotype and resulted in a small amino acid change (Asp-Phe to Ile at position 198) in the protein. The grandparents, who had no signs of TSC, tested negative for the mutation. In a sporadic patient (T5913), 9 bp were deleted in exon 15, also leading to a different protein product (Cys-Lys-Ile-Pro to Ser at aa position 586). Both parents tested negative for the mutation. Biological parenthood was confirmed for both in frame deletions. All the other deletions led to a premature stop codon. Nonsense mutations were detected in 11 cases; R692X was present in four sporadic cases and in one family. Three insertions were identified: a single base pair substitution in exon 7 and exon 10 in familial cases, and a duplication of 28 bp in exon 17 in a sporadic patient. A substitution at a splice site (bp position 432-1) was detected in a sporadic patient. A substitution at a splice site in four sporadic cases and in one family. Three insertions were identified: a single base pair substitution in exon 7 and exon 10 in familial cases, and a duplication of 28 bp in exon 17 in a sporadic patient. A substitution at a splice site (bp position 432-1) was detected in a sporadic patient. A substitution at a splice site in four sporadic cases and in one family. Three insertions were identified: a single base pair substitution in exon 7 and exon 10 in familial cases, and a duplication of 28 bp in exon 17 in a sporadic patient. A substitution at a splice site (bp position 432-1) was detected in a sporadic patient. A substitution at a splice site in four sporadic cases and in one family. 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CLINICAL MANIFESTATIONS IN PATIENTS WITH THE RECURRENT MUTATION R692X

The mutation R692X was present in four sporadic TSC patients and in two patients from the same family. The clinical data of these six patients are summarised in table 4. Almost all patients have a history of epilepsy. No renal lesions were detected, but only two patients are older than 15, so these results could be biased by the later onset of cysts and angiomylipomas. All other symptoms were scored at least once. The patients with the R692X mutation do not share an obviously similar phenotype.

Discussion

After screening the 21 coding exons of the TSC1 gene, 29 mutations were detected, all of

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Table 2 Missense and silent changes in the TSC1 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Code</th>
<th>nt change</th>
<th>aa change</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>T5768</td>
<td>374A→C</td>
<td>E51D</td>
</tr>
<tr>
<td>7</td>
<td>T1486</td>
<td>789C→T</td>
<td>R1908*</td>
</tr>
<tr>
<td>11</td>
<td>T10383</td>
<td>429C→T</td>
<td>S403</td>
</tr>
<tr>
<td>14</td>
<td>T2083</td>
<td>1556A→G</td>
<td>E445*</td>
</tr>
<tr>
<td>15</td>
<td>T1250</td>
<td>2155C→T</td>
<td>H732Y*</td>
</tr>
<tr>
<td>22</td>
<td>T1219</td>
<td>3050C→T</td>
<td>A943*</td>
</tr>
</tbody>
</table>

*Confirmed polymorphism: E445, H732Y and A943 were reported before. nt = nucleotide, aa = amino acid.

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Table 3 Overall summary of clinical features of all patients with mutations in the TSC1 gene. A distinction has been made between patients with a stop mutation and an in frame deletion

<table>
<thead>
<tr>
<th>Stop mutation</th>
<th>In frame deletion</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Facial angiofibroma</td>
<td>17/29</td>
<td>3/3</td>
</tr>
<tr>
<td>Ungual fibroma</td>
<td>10/26</td>
<td>2/3</td>
</tr>
<tr>
<td>Hypomelanotic macule</td>
<td>23/26</td>
<td>4/4</td>
</tr>
<tr>
<td>Subependymal nodules</td>
<td>24/28</td>
<td>3/4</td>
</tr>
<tr>
<td>Cortical tuber</td>
<td>10/28</td>
<td>2/4</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>23/30</td>
<td>2/4</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>13/27</td>
<td>1/3</td>
</tr>
<tr>
<td>Renal cyst</td>
<td>4/26</td>
<td>0/4</td>
</tr>
<tr>
<td>Renal angiomyolipoma</td>
<td>1/26</td>
<td>0/4</td>
</tr>
<tr>
<td>Cardiac rhabdomyoma</td>
<td>5/24</td>
<td>1/4</td>
</tr>
</tbody>
</table>

---

Table 4 Clinical features of patients with mutation R692X

<table>
<thead>
<tr>
<th>T1197a</th>
<th>T1197b</th>
<th>T3838</th>
<th>T3908</th>
<th>T5210</th>
<th>T10816</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56</td>
<td>15*</td>
<td>14</td>
<td>54</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Facial angiofibroma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>Ungual fibroma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>Hypomelanotic macule</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4/6 (67%)</td>
</tr>
<tr>
<td>Subependymal nodules</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>Cortical tuber</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Renal cyst</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Renal angiomyolipoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Cardiac rhabdomyoma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>2/6 (33%)</td>
</tr>
</tbody>
</table>

* Died aged 15 of giant cell astrocytoma.
them small changes. The only mutation detected by Southern blot analysis was the substitution of a C for T in a TaqI (TCGA) restriction site, resulting in a stop codon. Since four different restriction enzymes have been used to test a selection of our TSC patients on Southern blots, it is unlikely that large abnormalities disrupting the TSC1 gene have remained undetected. Previous mutation studies in the TSC2 gene have shown a diverse mutational spectrum including large rearrangements, deletions, insertions, and nonsense and missense mutations. In the TSC2 gene, approximately 10% of the mutations detected so far were large deletions, often resulting in disruption of the neighbouring PKD1 gene as well. A possible explanation for the lack of large mutations in TSC1 may be the presence of unknown neighbouring or intragenic genes that are essential for embryonic development and survival. Although all the mutations detected in the TSC1 gene were small, we could not confirm any nonsense change as the disease causing mutation. Only one missense mutation in TSC1 has been reported before by Jones et al (personal communication), but this de novo change in the gene appeared not to be the disease causing mutation. Conversely, a number of missense mutations have been reported in the TSC2 gene. It remains to be explained why the mutational spectrum of the TSC1 and TSC2 genes is different. Despite the differences, most of the mutations in either TSC1 or TSC2 lead to a truncated protein, which is in concordance with a loss of function mechanism. Results obtained by interaction studies indicate that hamartin and tuberin function as a complex, which supports the phenotypic overlap observed between TSC patients with either a TSC1 or TSC2 mutation.

So far we have detected a mutation in the TSC1 gene in 13% of our unrelated TSC collection, screening all of the coding region of the gene by SSCP analysis. It is likely that this technique fails to detect all of the mutations and the promoter region has not been tested yet. We only detected the disease causing mutation in two out of three of our families which were clearly linked to chromosome 9. This number is too small to give an indication of the ratio of undetected mutations in the TSC1 gene, but we expect that for the whole group of TSC patients, the majority of the mutations will be found in the TSC2 gene. We did not observe a significantly larger number of TSC1 mutations in our familial cases than in the sporadic population, as was found in a recent study, although it is possible that some of our patients were misclassified as sporadic, because in only half of these cases was material from both parents available.

We found a clustering of mutations in exons 15 and 17, in which 14 out of 29 identified mutations were present. The high mutation rate in exon 15 had already been observed when the TSC1 gene was identified and can be partially explained by the size of the exon (17% of the coding region). Furthermore, the high proportion of recurrent mutations detected in exons 15 and 17 suggests that part of these exons are particularly prone to nucleotide changes.

The truncating mutations as compared to the in frame deletions in the TSC1 gene did not show an obvious difference in clinical phenotype in the patients, suggesting that there is no clear correlation between the nature of a TSC1 mutation and the clinical phenotype. The six patients with the recurrent mutation R692X also displayed a wide range of clinical symptoms. This is comparable to the clinical differences detected within families with TSC. The phenotypic differences in TSC patients are more likely to be caused by mechanisms such as a second hit, somatic mosaicism, and modifying genes. The latter has also been proposed to contribute to the complex phenotype in the comparable “monogenic” disease neurofibromatosis 1 (NF1). In conclusion, we found no support for a different phenotypic spectrum in patients with a mutation in the TSC1 gene compared to the overall population of TSC patients.

The first two authors contributed equally to this work. We are grateful to the patients and their families for their participation, to the clinicians and the TSC Working Group Rotterdam for referral of the TSC patients, and to Professor H Galjaard for continuous support. One of the authors (V van Slegtenhorst) was funded by the Dutch Organization for Scientific Research (NWO), the Dutch Pravention Fund (grant number 28-1827), and the Dutch Kidney Foundation (grant number C93.1313).


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