Clinical genetic study in patients with tuberous sclerosis complex

Estudio clínico genético en pacientes con complejo de esclerosis tuberosa

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Abstract

Introduction: Tuberous sclerosis complex (TSC) is a multisystem autosomal dominant disease caused by mutations in the tumor suppressor genes TSC1 or TSC2. Objective: To characterize clinically and genetically patients diagnosed with TSC. Patients and method: Descriptive study of clinical records from a pediatric neuropsychiatry department of 42 patients diagnosed with TSC and genetic study of 21 of them. The exon 15 of the TSC1 gene and exons 33, 36 and 37 of the TSC2 gene were amplified by polymerase chain reaction and sequenced. The relationship between the mutations found with the severity and clinical evolution were analyzed. Results: In 61.9% of the patients the symptoms began before 6 months of age. The most frequent initial manifestations of TSC were new onset of seizures (73.8%) and the detection of cardiac rhabdomyomas (16.6%). During the evolution of the disease all patients had neurological involvement; 92.9% had epilepsy. All patients presented hypomelanotic spots, 47.6% facial angiofibromas, 23.8% Shagreen patch, 47.6% heart rhabdomyomas and 35.7% retinal hamartomas. In the genetic study of 21 patients, two heterozygous pathogenic mutations in TSC1 and one in TSC2 genes were identified. The latter had a more severe clinical phenotype. Conclusions: Neurological and dermatological were the most frequent manifestations in patients with TSC. Two pathogenic mutations in TSC1 and one in TSC2 genes were identified. The patient with TSC2 mutation manifested a more severe clinical phenotype.
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Patients and Method

Study was approved by the Ethics Committee of the Health Service of Santiago. Adult patients and parents of pediatric patients were informed about this study and were invited to participate, after which they signed an informed consent document.

Clinical characterization

Description of 42 patients with TSC receiving care at the Child Neuropsychiatry Service of the San Borja Arriaran Hospital from 1989 to 2013. Clinical history was analyzed and complementary examinations performed (electroencephalogram [EEG], radiological, ultrasonographic and ophthalmologic studies). The data collected from the clinical records were: gender, family history of TSC, age that symptoms or signs of the disease were first present, and symptoms or signs that motivated the consultation to child neurologist. We also included the characterization of neurological involvement, specifying the level of psychomotor development, autistic behaviors and epilepsy. A detailed characterization of the epileptic phenotype was performed, including the average age of onset of seizures, type of epileptic syndrome and response to antiepileptic drugs. Characteristic lesions of the disease found in neuroimaging and other affected organs were analyzed.

Genetic analysis

The genetic analysis was performed in 21 patients who accepted to participate in the study, 2 of them were related (mother and child). A peripheral blood sample was obtained to extract DNA and exon 15 of TSC1 gene and exons 33, 36 and 37 of TSC2 gene were amplified by PCR. The products amplified by PCR were sent to the biotech company Macrogen (Seoul, Korea).

sy, cognitive deficits, behavioral disorders, autism and different types of brain lesions. In more than 88% of the patients cortical tufts are produced, varying in size and number, the type that persist throughout life that do not become malignant. These lesions have been associated with the development of refractory epilepsy and intellectual disability. Cutaneous involvement is due to hypopigmented macules in 90-98% of patients and facial angiofibroma in 75%, among other lesions. In the heart the main lesion found corresponds to rhabdomyoma, usually benign and with tendency to regress completely during childhood. It is usually asymptomatic, although it may be associated with cardiac arrhythmias, obstruction to the outflow tract and cardioembolic disease. Pulmonary involvement corresponds to lymphangioleiomyomatosis, which mainly affects women. Recent studies suggest that lung involvement may increase with age, affecting 80% of women with TSC by 40 years of age. Renal involvement occurs in 80% of cases and includes angiomyolipomas (AML) and renal cysts. AMLs tend to increase in number and size with age. Renal complications are the leading cause of death in TSC, whether due to AML hemorrhage or renal failure. Different types of lesions in the liver, such as lipomas, hamartomas, fibromas and AML, may be found that behave in a similar way as in the kidney, but with a slower growth and no risk of death from bleeding.

TSC presents an autosomal dominant inheritance pattern with variable expressivity, as well as genetic and allelic heterogeneity. It is caused by mutations in tumor suppressor genes TSC1 and TSC2. The TSC1 gene is located on chromosome 9 in the 9q34 band and codes for the hamartin protein ~ 53kb. The TSC2 gene is located on chromosome 16 in the 16p13.3 band and codes for the tuberin protein 40.7kb. There are more mutations reported in TSC2 than in TSC1, with at least 405 mutations in TSC1 and 1,128 in TSC2. In 15% of TSC cases it is not possible to identify a mutation using conventional sequencing methods. There is a higher concentration of mutations in exons 15 of TSC1 and 33, 36 and 37 of TSC2. Mutations in TSC2 are 5 times more frequent than in TSC1 in sporadic cases. In familiar cases the ratio is 1: 1.

For the occurrence of TSC it is required that one of the 2 alleles of TSC1 or TSC2 is inactive, although for some tissues, the second allele (loss of heterozygosity) is also inactivated. There is more evidence of loss of heterozygosity in renal AML and less in subcellular giant cell astrocytomas (SEGA). There are tumors in which no loss of heterozygosity (cortical tufts and ungual fibromas) has been observed, suggesting the existence of other mechanisms involved, such as haploinsufficiency. The proteins hamartin and tuberin form a cytoplasmic dimer that acts through the Ras homolog enriched in brain (Rheb) protein, which positively regulates its GTPase activity and diminishes the stimulation of mTOR. MTOR is a serine/threonine kinase that fulfills central regulatory functions of many signaling pathways, including regulation of proliferation, cell size/growth, translation, metabolism, autophagy, angiogenesis and survival in response to nutrient availability (glucose and amino acids). Several studies that have analyzed the relationship between genotype and phenotype have described that patients with mutations of the TSC2 gene evolve more severe symptoms.

The aim of this study was to analyze the relationship between the mutations found with the clinical characteristics of the patients. This is the first clinical-genetic study in TSC carried out in Chile.
Results

General characteristics
We studied 42 patients with clinical diagnosis of TSC. Of these, 24 (57%) were male and 18 (43%) were female. Only 6 patients (14.3%) had a family history of TSC. In 7 (17%) of the patients symptoms and signs of TSC started earlier than one week old, 26 (61.9%) patients started before 6 months of age, and in 35 (83.3%) of the patients clinical manifestations were evident before 12 months of life. In 31 patients (73.8%) the initial manifestation of the disease was epileptic seizures and in 7 patients (16.6%) the presence of cardiac rhabdomyomas was present. Two patients (4.8%) consulted for delayed psychomotor development and 2 (4.8%) for skin lesions.

Neurological manifestations
All the patients presented neurological compromise, including delayed psychomotor development, epilepsy and/or brain lesions characteristic of TSC. Delayed psychomotor development was presented in 31 patients (73.8%); in 25 (59.5%) of them delayed psychomotor development was severe, whereas in 6 (14.3%) it was mild. Twelve patients (28.6%) presented autistic behaviors and 39 (92.9%) evolved with epilepsy (Table 1). The mean age at onset of epileptic seizures was 11.6 months (range: 2-84 months). Of the 39 patients who presented epilepsy, 19 (48.7%) developed infantile spasms and 2 (5.1%) had Lennox-Gastaut syndrome, the final evolution of all of them was a symptomatic, focal epileptic syndrome (Table 1). In most cases epilepsy was difficult to manage, requiring multiple changes in antiepileptic regimens and therapies of 2 or 3 associated drugs.

Forty of the 42 patients had neuroimaging. 23 of 40 patients underwent cerebral computed tomography (CT) and nuclear magnetic resonance (MRI) in the brain, 12 of 40 had CT alone and 5 of 40 had MRI alone. Overall, neuroimaging findings were found to be compatible with TSC in 38 patients. Subependymal nodules (SEN) were observed in 16 patients, subependymal calcifications in 17 patients, cortical tubers in 14 patients and no alterations were observed in 6 patients.

All patients who had a brain MRI presented at least one type of lesion characteristic of TSC. In the MRI of the brain, SEN was observed in 20 patients, cortical tubers in 22 patients, SEGA in 2 patients and white matter lesions characteristic of TSC in 4 patients (Table 1). In 5 of the 23 patients with CT and MRI of the brain, CT was normal and MRI presented pathological findings of TSC (Figure 1). There were no patients with altered CT scan and normal MRI.

Extra-neurological manifestations
All patients underwent echocardiography, renal ultrasound, dermatological and ophthalmological evaluations as part of the follow-up of TSC. Table 2 shows a summary of the extraneurological manifestations observed in the patients.

Cardiac compromise
Cardiac rhabdomyomas were observed in 20 patients (47.6%), 2 of whom were diagnosed during antenatal period and 5 in the neonatal period. Of the 42 patients, 2 (4.7%) had neonatal heart failure due to the presence of rhabdomyomas, one patient (2.4%) had bradycardia at birth and 3 (7.1%) had Wolff-White syndrome. One patient had obstruction of the left ventricular outflow tract (LVOT) secondary to rhabdomyomas, and cardiac surgery was performed at one month of age (Table 2).
Renal compromise

Of the 42 patients studied, 12 (28.6%) presented TSC-like lesions on the renal ultrasonography, 7 of them (16.7%) had AML and 6 (14.3%) presented renal cysts. Two of the 6 patients with renal cysts (33.3%) presented a suggestive pattern of polycystic kidney disease associated with TSC (Table 2).

Dermatological compromise

All patients had hypomelanotic macules and 5 of them (11.9%) presented lesions in “confetti” pattern. Facial angiofibromas were observed in 20 of the 42 patients (47.6%), Shagreen patches in 10 patients (23.8%), cephalic fibrosis plaque in 4 patients (9.5%), and ungual fibroids in 6 (14.3%) (Table 2).

Table 1. Neurological manifestations and neuroimaging findings in patients with TSC

<table>
<thead>
<tr>
<th>Neurological manifestations</th>
<th>Patients N (%)</th>
<th>Neuroimaging</th>
<th>Patients N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with neurological manifestations</td>
<td>42</td>
<td>Total neuroimaging patients</td>
<td>40</td>
</tr>
<tr>
<td>Delayed psychomotor development</td>
<td>31 (73.8)</td>
<td>Neuroimaging compatible CET</td>
<td>38</td>
</tr>
<tr>
<td>Mild delayed psychomotor development</td>
<td>6 (14.3)</td>
<td>Total patients with TAC</td>
<td>35</td>
</tr>
<tr>
<td>Severe delayed psychomotor development</td>
<td>25 (59.5)</td>
<td>TAC normal</td>
<td>6</td>
</tr>
<tr>
<td>delayed psychomotor development predominated language</td>
<td>14 (33.3)</td>
<td>Subependymary nodules</td>
<td>16</td>
</tr>
<tr>
<td>Autistic Spectrum Disorder</td>
<td>12 (28.6)</td>
<td>Subependymal calcifications</td>
<td>17</td>
</tr>
<tr>
<td>Epilepsia</td>
<td>39 (92.9)</td>
<td>Types cortical</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Epilepsy</th>
<th>Patients N (%)</th>
<th>Total of patients with MRI</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age onset of crisis (months)</td>
<td>11.6</td>
<td>Normal RMN</td>
<td>0</td>
</tr>
<tr>
<td>Childhood spasms</td>
<td>19 (48.7)</td>
<td>Subependymary nodules</td>
<td>22</td>
</tr>
<tr>
<td>Lennox-Gastaut Syndrome</td>
<td>2 (5.1)</td>
<td>Types cortical</td>
<td>2</td>
</tr>
<tr>
<td>Symptomatic focal epileptic syndrome</td>
<td>39 (100)</td>
<td>SEGA</td>
<td>2</td>
</tr>
<tr>
<td>Percentage in parentheses. TSC: tuberous sclerosis complex; MRI: nuclear magnetic resonance; SEGA: subependymal giant cell astrocytoma; CT: computerized axial tomography.</td>
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</table>

Table 2. Extranurologic manifestations in patients with TSC

<table>
<thead>
<tr>
<th>Cardiac compromises</th>
<th>Patients N (%)</th>
<th>Dermatological compromises</th>
<th>Patients N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac rhabdomyomas</td>
<td>20/42 (47.6)</td>
<td>Hypomelanotic macules</td>
<td>42/42 (100)</td>
</tr>
<tr>
<td>Antenatal diagnosis</td>
<td>2 (10)</td>
<td>Facial angiofibromas</td>
<td>20/42 (47.6)</td>
</tr>
<tr>
<td>Neonatal diagnosis</td>
<td>5 (25)</td>
<td>Shagreen Patch</td>
<td>10/42 (23.8)</td>
</tr>
<tr>
<td>Neonatal cardiac insufficiency</td>
<td>2/42 (4.7)</td>
<td>Cephalic fibrous plaque</td>
<td>4/42 (9.5)</td>
</tr>
<tr>
<td>LVOT Obstruction</td>
<td>1/42 (2.4)</td>
<td>Ungual fibromas</td>
<td>5/42 (12)</td>
</tr>
<tr>
<td>Brachyacardia</td>
<td>1/42 (2.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolff-Parkinson-White Syndrome</td>
<td>3/42 (7.1)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Renal compromises</th>
<th>Patients N (%)</th>
<th>Ocular compromises</th>
<th>Patients N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Findings of TSC in renal ultrasound</td>
<td>12/42 (28.6)</td>
<td>Retinal hamartomas</td>
<td>15/42 (35.7)</td>
</tr>
<tr>
<td>Angiomyolipomas</td>
<td>7/42 (16.7)</td>
<td>Nonatal diagnosis</td>
<td>1/15 (6.7)</td>
</tr>
<tr>
<td>Renal cysts</td>
<td>6/42 (14.3)</td>
<td>Diagnosis &lt; 1 year</td>
<td>8/15 (53.3)</td>
</tr>
<tr>
<td>Suspected Polycystic Kidney Disease</td>
<td>2/6 (33.3)</td>
<td>Acromic patch</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

TSC: tuberous sclerosis complex; LVOT: left ventricular outflow tract.
Eye compromise

Retinal hamartomas were observed in 15 of the 42 patients (35.7%). In one of the 15 patients (6.7%) with retinal hamartomas the diagnosis was during neonatal period, whereas in 8 (53.3%) these lesions were detected during the first year of life (Table 2).

Genetic study

In the search for mutations in exons 15 of TSC1 and 33, 36 and 37 of TSC2 performed in 21 patients, mutations were detected in 4 of them. These mutations have been previously reported in the literature. The results of the genetic study together with the clinical characteristics of these patients are summarized in Table 3.

In two related patients (mother and child) the same mutation was detected, corresponding to a nonsense mutation (c.1525C>T: p.R509*). In one patient the c.1997+1G>C mutation was detected at a splicing site of the intron. Finally, in a patient with a severe phenotype, the mutation c.4928A>G (p.N1643S) was detected corresponding to a asparagine to serine change at codon 1643. The most severe phenotype was observed in the patient with mutation in the gene TSC2.

Discussion

The aim of this work was to know the clinical characteristics of Chilean pediatric patients diagnosed with TSC in a Child Neuropsychiatry Service, to explore the genetic causes of this disease and the relationship between mutations and clinical characteristics. Considering that TSC is an autosomal dominant genetic disease with variable expressivity and incomplete penetrance, the expected phenotypes should be diverse. On the other hand, since the analyzed population was recruited in a Child Neurology Service, the observed phenotypes have a greater severity bias.

In our series of 42 patients, only 15% had a family history of TSC. This percentage is lower than described in literature, which reports that about a third of the cases would have a family history. This could be explained by the underdiagnosis in the parents due to asymptomatic manifestations, such as cardiac rhabdomyomas that reverted during childhood, retinal hamartomas or SEN.

In 62% of the patients the initial signs and symptoms were detected before the 6 months of life and in 17% the detection was before one-week old, which is earlier than reported in the literature.

The initial manifestations of TSC were seizures in 73% of the cases, cardiac rhabdomyomas in 17%, delayed psychomotor development in 5% and skin lesions in 5%. During their evolution, all the patients presented some manifestation of neurological compromise. 73.8% of the patients presented delayed psychomotor development, being severe in 59.5% of them. 28.6% of the patients presented autistic behaviors and 92.9% evolved with epilepsy. The presence of autistic behaviors was associated with the coexistence of epilepsy with early onset and being difficult to manage, which is in agreement with what has been described in the literature.

The mean age at onset of epileptic seizures was 11.6 months, ranging from 1 to 84 months. Another series of cases shows average age of onset at 29 months. In both, in most patients the onset of crisis was earlier than 1 year of age. 48.7% of the patients with epilepsy evolved with infantile spasms, which is higher than reported in the literature, which describes a frequency of infantile spasms between 20 and 37%.

5.1% of patients with epilepsy evolved to Lennox-Gastaut syndrome, which is very similar to that described in the literature. In addition to early onset, epilepsy was difficult to manage, requiring multiple changes in antiepileptics and administration of 2 or 3 associated drugs. All patients with epilepsy evolved to focal epileptic syndrome.
66.7% of the patients had brain MRI and all resulted in characteristic findings of TSC. Of these, 19% had a previous CT scan without signs of TSC; this emphasizes the importance of MRI for a good diagnosis. It is important to mention that the current international recommendations suggest the realization of brain MRI every 1-3 years in patients with asymptomatic TSC, and if they present a large SEGAs, it is suggested to control it with more frequent MRI32. It is reported in the literature that 80% of the patients with TSC have SEN, 90% have cortical tubers, and 5-15% have SEGAs during evolution33.

Cardiac rhabdomyomas are the main characteristic of this disease in the fetal and neonatal periods34, and are rarely observed in patients who do not have TSC33. In our series, 49% of the patients had cardiac rhabdomyomas on echocardiography. In 10% of them, rhabdomyomas were searched in the antenatal period and in 25% in the neonatal period. One patient had an obstruction of LVOT, which required cardiac surgery at one month of age, with subsequent good evolution. It is common for large lesions to cause obstruction of LVOT or cardioembolic disease34. One patient had bradyarrhythmia at birth and three patients had Wolff-Parkinson-White syndrome; in 2 of them, the diagnosis was neonatal and in one tachyarrhythmia was detected in childhood. It is important to emphasize not only the need for cardiological follow-up with echocardiography until the regression of rhabdomyomas, but also with electrocardiogram at all ages to detect heart conduction defects early, as occurred in one of our patients32.

29% of the patients had lesions characteristic of TSC on renal ultrasounds, 17% had AML and 15% had renal cysts. Of the latter, 2 patients had a suggestive pattern of polycystic kidney disease associated with TSC. None of the patients reported secondary involvement of renal functions with the presence of AML. The literature describes that renal complications are the most frequent cause of death related to TSC, with the identification of multiple and bilateral AMLs in 70-90% of adult patients. The frequency is much lower in children, reporting up to 16% in patients younger than 2 years34. The low percentage of patients with renal impairment reported in our series is understood as it corresponds to pediatric patients whose disease has not yet developed renal manifestations.

In our series, dermatological involvement was present in 100% of the patients. All had hypomelanotic macules, 46% facial angiofibromas, 22% Shagreen patch, 10% cephalic fibrous plaque, 10% “confetti” lesions and 12% nail fibromas. Our findings are similar to those described in literature, taking into consideration that hypomelanotic macules may be the only cutaneous manifestation of TSC in small patients3.

In our series, 37% of patients had retinal hamartomas. In 7% of them, the diagnosis was neonatal, whereas in 53% these lesions were detected during the first year of life. Our findings coincide with those described in the literature, emphasizing the importance of ophthalmological evaluation during the first year of life to support early diagnosis3.

In the genetic study performed in 21 patients, 3 previously described mutations were identified. The same mutation was detected in 2 related cases. But this study only included a limited number of analyzed exons. In 4 patients, sequence variations (described as pathogenic mutations) were detected, 3 in the TSC1 gene and one in the TSC2 gene. These mutations, reported at http://www.lovd.nl, correspond to a nonsense mutation c.1525C>T (p.R509*) in the TSC1 gene found in the 2 related patients (mother and child), splice donor site mutation c.1997+1G>C also found in the TSC1 gene of a patient, and a mutation in the TSC2 c.4928A>G gene (p.N1643S) in exon 37 (Table 3). The mutation c.1525C>T (p.R509*) corresponds to the code CM971518 of the Human Gene Mutation Database (HGMD) and has been reported 28 times in both familial and sporadic cases (http://www.lovd.nl/TSC1). The mutation of the splicing donor site c.1997+1G>C is not reported in HGMD, and has only been described in one patient, published directly at http://www.lovd.nl/TSC1.

Regarding the mutation c.4928A>G (p.N1643S) in exon 37 of the TSC2 gene, 3 pathogenic mutations in the same codon have been reported in HGMD, none of which corresponds to the variation detected in our study.

The literature describes a mutation detection rate ranging from 75-90%, when the simultaneous analysis is performed of all exons coding for the TSC1 and TSC2 genes, in addition to the surrounding regions35. The detection rate of mutations in our study was 19%, which is not low if we consider that only 4 exons were analyzed. This is explained by a slightly higher concentration of mutations in these exons, which was the reason why they were selected for this analysis34.

Several studies have analyzed the relationship between genotype and phenotype in TSC, demonstrating that mutations in the TSC2 gene are manifested with greater severity than mutations in TSC1. Specifically, they have been associated with a high frequency of intellectual disability, severe onset epilepsy, cortical tubers and extensive renal involvement31,12,36. In our study we identified only one pathogenic mutation in TSC2 (c.4928A>G (p.N1643S)) in a patient with a particularly severe clinical course, presenting infantile spasms since 5 months of age which were very difficult to manage. He also presented several types of
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References


Ethical Responsibilities

Protection of people and animals: The authors state that no experiments have been performed on humans or animals for this research.

Confidentiality of data: The authors state that they have followed the protocols of their work center on the publication of patient data.

Privacy rights and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

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Conflict of interest

The authors declare that they have no conflict of interest.

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