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ORIGINAL ARTICLE

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.

Keywords:
TSC; SEGA;
Epilepsy;
Children;
Tubers;
Hamartoms.

Introduction

Tuberous sclerosis complex (TSC) is a genetic-based neurocutaneous disorder. It is characterized by multisystemic involvement, with formation of hamartomas in various organs including: brain, heart, skin, eyes, kidney, lung and liver[1]. The reported incidences in different studies are 1/6,000 - 1/10,000 live births[2-4]. The TSC phenotype is very diverse[5,6] and clinical expression is closely related to age.

More than 85% of the patients present central nervous system (CNS) alterations, including epilep-

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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[7-10]. Cutaneous involvement is due to hypopigmented macules in 90-98% of patients and facial angiofibroma in 75%, among other lesions[5,11-14]. 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[15,16]. 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[17]. 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[18-21]. 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[22].

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 tuber protein 40.7kb[23]. There are more mutations reported in TSC2 than in TSC1, with at least 405 mutations in TSC1 and 1,128 in TSC2[23]. 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[24]. Mutations in TSC2 are 5 times more frequent than in TSC1 in sporadic cases. In familiar cases the ratio is 1: 1[24].

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[25]. 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[26,27]. 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[11,12,28].

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.

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[29] 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).

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).

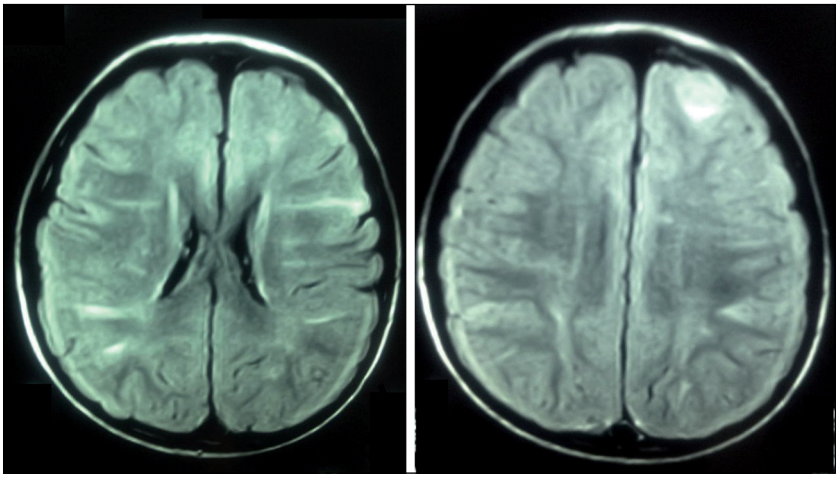

Figure 1. Brain magnetic resonance imaging (MRI) in a patient with tuberous sclerosis complex (TSC). MRI axial cut of cerebral (FLAIR) in a patient with TSC, where hyperintense radial bands are observed in both hemispheres **(A)** and cortical tuft in the left frontal lobe **(B)**.

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

Neurological manifestations	Patients N (%)	Neuroimaging	Patients N
Patients with neurological manifestations	42	Total neuroimaging patients	40
Delayed psychomotor development	31 (73.8)	Neuroimaging compatible CET	38
Mild delayed psychomotor development	6 (14.3)	Total patients with TAC	35
Severe delayed psychomotor development	25 (59.5)	TAC normal	6
delayed psychomotor development predominated language	14 (33.3)	Subependymary nodules	16
Autistic Spectrum Disorder	12 (28.6)	Subependymal calcifications	17
Epilepsia	39 (92.9)	Types cortical	14
Epilepsy	**Patients N (%)**	Total of patients with MRI	28
Total of patients with epilepsy	39	Normal RNM	0
Average age onset of crisis (months)	11.6	Subependymary nodules	20
Childhood spasms	19 (48.7)	Types cortical	22
Lennox-Gastaut Syndrome	2 (5.1)	SEGA	2
Symptomatic focal epileptic syndrome	39 (100)	White matter lesions	4

Percentage in parentheses. TSC: tuberous sclerosis complex; MRI: nuclear magnetic resonance; SEGA: subependymal giant cell astrocytoma; CT: computerized axial tomography.

Table 2. Extranurologic manifestations in patients with TSC

Cardiac compromises	Patients N (%)	Dermatological compromises	Patients N (%)
Cardiac rhabdomyomas	20/42 (47.6)	Hypomelanotic macules	42/42 (100)
Anten Antenatal diagnosis	2 (10)	Facial angiofibroma	20/42 (47.6)
Nonatal diagnosis	5 (25)	Shagreen Patch	10/42 (23.8)
Neonatal cardiac insufficiency	2/42 (4.7)	Cephalic fibrous plaque	4/42 (9.5)
LVOT Obstruction	1/42 (2.4)	Ungual fibromas	5/42 (12)
Bradycardia	1/42 (2.4)		
Síndrome Wolff-Parkinson-White	3/42 (7.1)		
Renal compromises	**Patients N (%)**	**Ocular compromises**	**Patients N (%)**
Findings of TSC in renal ultrasound	12/42 (28.6)	Retinal hamartomas	15/42 (35.7)
Angiomyolipomas	7/42 (16.7)	Nonatal diagnosis	1/15 (6.7)
Renal cysts	6/42 (14.3)	Diagnosis < 1 year	8/15 (53.3)
Suspected Polycystic Kidney Disease	2/6 (33.3)	Acromic patch	0 (0)

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

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 3. Clinical and genetic characteristics of patients with TSC

Gene	Sequence variation	FH	Age at diagnosis (m)	Initial clinical presentation	Evolution of epilepsy	CT	SEN	SEGA	DSD	ASD	HM	FA	SP	CR	RH
TSC2	c.4928A>G (p.N1643S)	N	5	Childhood spasms	FE	N	Y	Y	Y	Y	Y	Y	Y	N	Y
TSC1	c.1525C>T (p.R509*)	N	36	Absence crisis	FE	Y	N	N	N	N	Y	N	Y	N	N
TSC1	c.1525C>T (p.R509*)	Y	6	Childhood spasms	FE	Y	N	N	Y	N	Y	N	N	N	N
TSC1	c.1997+1G>C	N	36	Focal crises	FE	Y	Y	N	N	N	Y	Y	N	Y	N

FA: facial angiofibromas; SEGA: subependymal giant cell astrocytoma; TSC: tuberous sclerosis complex; DPD: delayed psychomotor development; FE: focal epilepsy; FH: family history of TSC; RH: retinal hamartomas; M: months; HM: hypomelanotic macules; SEN: subependymal nodules; SP: Shagreen patch; CR: cardiac rhabdomyomas; CT: cortical tufts; ASD: autism spectrum disorder; Y: Yes; N: No.

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[8,24]. 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[6].

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[3].

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[10]. 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%[10,30,31]. 5.1% of patients with epilepsy evolved to Lennox-Gastaut syndrome, which is very similar to that described in the literature[10]. 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 emphazises 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 SEGA, it is suggested to control it with more frequent MRI[32]. It is reported in the literature that 80% of the patients with TSC have SEN, 90% have cortical tubers, and 5-15% have SEGA during evolution[33].

Cardiac rhabdomyomas are the main characteristic of this disease in the fetal and neonatal periods[34], and are rarely observed in patients who do not have TSC[33]. 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 disease[34]. One patient had bradycardia 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 patients[32].

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 years[34]. 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 patients[3].

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, emphazising the importance of ophthalmological evaluation during the first year of life to support early diagnosis[3].

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 regions[3,35]. 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 analysis[24].

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 involvement[3,11,12,36]. 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

crises including tonic crises, absences and tonic-clonic seizures, requiring multiple antiepileptic drug regimens. A predominantly global delayed psychomotor development in language was present and this is one of the 2 patients in our series who presented SEGA, associated to multiple SEN. Retinal hamartomas and extensive dermatological compromise were also present.

Although some relationships between genotype and phenotype have been established, the clinical spectrum in TSC is highly variable. In our study we could only compare the phenotypes of 2 patients with the same mutation. They were mother and child, and both presented the nonsense mutation c.1525C>T (p.R509*) in TSC1, but they manifested very different phenotypes. The mother had normal IQ, was autovalent and had a history of non-refractory epilepsy. Her son, on the other hand, started infantile spasms at 6 months of age. Treatment with vigabatrin was shown to be unresponsive. It then evolved with different types of seizures, requiring polytherapy of antiepileptic drugs with little response. He presented delayed psychomotor development predominantly in language and multiple cortical tubers.

This is the first study in Chile that clinically and genetically analyzed patients with TSC. We hope that in the future new studies will be carried out like this one with a greater number of TSC patients and with a greater number of exons.

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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