Two novel TSC2 mutations in pediatric patients with tuberous sclerosis complex

Case report
Shan Gao, MD<sup>a,b</sup>, Zhiling Wang, MD<sup>a,b</sup>,*, Yongmei Xie, MD<sup>a,b</sup>

Abstract
Rationale: Tuberous sclerosis complex (TSC) is a rare autosomal dominant disorder. The TSC1 and TSC2 genes have been identified as pathogenic genes.

Patient concerns: In this report, we are discussing a novel frameshift mutation and a novel missense mutation in the TSC2 gene.

Diagnoses: The two cases discussed in this study met the latest diagnostic criteria for TSC published by the International Tuberous Sclerosis Complex Consensus Conference in 2012.

Interventions: High-throughput sequencing and multiplex ligation-dependent probe amplification (MLPA) were used to examine tuberous sclerosis complex (TSC)-related genes (TSC1 and TSC2) and their splicing regions using peripheral blood DNA from two probands in two families with TSC and to identify the genetic mutation sites. Amplification primers were designed for the mutation sites, and polymerase chain reaction and Sanger sequencing were used to verify the peripheral blood DNA sequences from the probands and their parents.

Outcome: Proband 1 had the c.1228 (exon 12)_c.1229 (exon 12) insG (p.L410RfsX11) heterozygous mutation in the TSC2 gene (chr16), which was a new frameshift mutation. Proband 2 had the c.4925G>A (exon 38) (p.G1642D) heterozygous mutation in the TSC2 gene (chr16), which was a new missense mutation.

Lessons: These two novel mutations may be pathogenic mutations for TSC, and their association with the disease needs to be further verified by mutant protein function cell model and animal model.

Abbreviations: ACTH = adrenocorticotrophic hormone, EDTA = ethylenediaminetetraacetic acid, EEG = electroencephalogram, GAP = GTPase activator protein, Indel = insertion–deletion, LM-PCR = ligase-mediated polymerase chain reaction, MLPA = multiplex ligation-dependent probe amplification, mTOR = mammalian target of rapamycin, S6K = S6 kinase, SEGAs = subependymal giant cell astrocortasmas, SENS = subependymal nodules, SIFT = scale invariant feature transform, SNP = single nucleotide polymorphism, T4PNK = T4 polynucleotide kinase, TSC = tuberous sclerosis complex.

Keywords: child, frameshift mutation, missense mutation, TSC2 gene, tuberous sclerosis complex

1. Introduction

Tuberous sclerosis complex (TSC; OMIM 613254) is a rare autosomal dominant disorder that is characterized by the formation of a hamartoma in multiple tissues and organs, which often include the brain, skin, heart, kidneys, lungs, and eyes. TSC can have diverse clinical manifestations, which are age-related. For example, epilepsy, mental retardation or regresion, autism, angiofibromatosis, shagreen patch, periungual fibromas, cardiac rhabdomyoma, renal angiomyolipoma, and lymphangioleiomyoma occur more frequently in adults than in children, whereas heart hamartomas are more common in children. The annual incidence in neonates is approximately 1:6000 to 1:10,000; sporadic cases account for approximately 2/3 of this total, which suggests a high rate of spontaneous mutations. Currently, the TSC1 and TSC2 genes, which are located on chromosomes 9q34.3 and 16p13.3, respectively, have been identified as pathogenic genes. TSC1 encodes a 130-kDa protein TSC1/hamartin and TSC2 encodes a 200-kDa protein TSC2/tuberin. The pathogenic mutations of the TSC1 and TSC2 genes include frameshift mutations, missense mutations, nonsense mutations, splicing mutations, deletions, and insertions. Neither gene has mutation hot spots. This article discusses a novel frameshift mutation and a novel missense mutation in the TSC2 gene.

2. Materials and methods
2.1. Subjects
The proband in Family 1 was male, 8 months and 3 days old, and ethnically Han from Leshan City, Sichuan Province. No abnormalities were reported during his mother’s pregnancy. The child was the second live-born progeny of 4 pregnancies (G4P2), was delivered at full term by cesarean section, and had no history of asphyxia. Abdominal and back discoloration was found 2 to 3 months after birth, and repeated involuntary
nodding- and embracing-like motions were observed 7 months after birth and occurred continuously for 1 to 3 minutes per episode at a rate of more than 10 episodes/day. At 8 months after birth, a video electroencephalogram (EEG) showed frequent multifocal (slow) and (multiple) spike (slow) waves, indicating that the clinical symptoms could have been due to seizures. Brain MRI showed multiple patchy abnormal signals in the bilateral hemispheres and bilateral multiple subependymal nodular shadows of the lateral ventricles, indicating the possibility of TSC (Fig. 1). Echocardiography showed septal and left intraventricular strong echoes (suggesting rhabdomyomas), and chest and abdominal noncontrast CT scans showed left renal cysts. The TSC gene detection analysis showed the c.1228 (exon 12).c.1229 (exon 12) insG (p.L410RfsX11) heterozygous mutation in the TSC2 gene (chr16). The development of the patient was delayed; the patient could lift his head and occasionally laugh upon stimulation when he was 4 months old and could sit with support, but he could not sit independently when he was 8 months old, which was the time of the examination. After the onset of the disease, the patient showed developmental retrogression, such as instability of the neck when held upright and an inability to laugh upon stimulation. The parents of the proband and the elder brother (3 years old) have no TSC-related symptoms.

The proband in Family 2 was male, 5 months and 3 days old, and ethnically Han from Jiangkou Township, Pingchang, Sichuan Province. No abnormalities were reported during the mother’s pregnancy. The child was the second live-born progeny of 2 pregnancies (G2P2), was delivered at full term by cesarean section, and had no history of asphyxia. In the second month after birth, discoloration of the forehead, neck, abdomen, and lower extremities was found and gradually increased. Repeated involuntary nodding- and embracing-like motions were observed 4 months after birth, and they occurred continuously for 1 to 2 minutes per episode at a rate of 3 to 4 episodes/day. At 5 months after birth, video EEG showed frequent multifocal sharp (slow), (multiple) spike (slow) waves, and slow waves, indicating that the clinical symptoms could have been due to seizures. The brain MRI showed various sizes of bilateral subependymal nodular shadows of the lateral ventricles and patchy abnormal signals in the deep bilateral temporal lobes, inferior horns of the lateral ventricle, frontal lobe, and parietal lobe, thus indicating the possibility of TSC (Fig. 2). Echocardiography showed right intraventricular occupancy (rhabdomyomas?), and chest noncontrast CT scanning showed pulmonary inflammation, right pleural constriction, and slight thickening. Ultrasound examination of the liver, gallbladder, pancreas, spleen, and urinary system revealed no abnormalities. TSC gene detection analysis showed the c.4925G>A (exon 38) (p. G1642D) heterozygous mutation in the TSC2 gene (chr16). The patient could lift his head when he was 3 months old, but he could not roll over at the time of the examination. After the onset of the disease, the patient showed developmental retrogression, such as instability of the neck when held upright and an inability to laugh upon stimulation. The parents of the proband and the elder sister have no TSC-related symptoms.
This study complied with the ethical standards issued by the Institutional Review Board and was approved by the Ethics Committee, West China Second Hospital, Sichuan University. The patients’ guardians signed informed consent forms and agreed to publish the examination results for medical research purposes.

2.2. Experimental methods

2.2.1. Peripheral blood genomic DNA extraction. After obtaining informed consent, 2 mL of peripheral blood was taken from the probands and the parents of the probands, with ethylenediaminetetraacetic acid (EDTA) added for anticoagulant treatment. Genomic DNA was extracted using the BloodGen Midi Kit (CW BIO, Beijing, China) according to the manual.

2.2.2. Next-generation sequencing. Based on the OMIM database and the literature, we designed Roche NimbleGen capture probes using the exon regions of the TSC-associated TSC1 and TSC2 genomic sequences for target gene whole exome capture.

Library preparation

a. Fragmentation of the genome: Genomic DNA was sheared to approximately 200 bp using a Cavoris instrument.

b. End repair: End repair of fragmented DNA was achieved using Klenow fragments, the T4 DNA polymerase, and the T4 polynucleotide kinase (T4PNK).

c. 3’-Adenosylation: The polymerase system added the A base at the 3’ end of the repair product obtained in step b.

d. Ligation of adapters: The T4 DNA ligase reaction system was used to ligate the adapter to the end of the repair product obtained in step b.

e. Amplification: The ligation product was amplified by 4 to 6 rounds of ligation-mediated polymerase chain reaction (LM-PCR).

f. Hybridization: The library was mixed with the probes in a hybridization system at 65°C for 60 to 68 hours of hybridization.

g. Washing of magnetic beads and DNA elution: After incubating the hybridization samples with streptomycin beads, the eluent was used to elute the DNA.

h. Amplification of the eluted product: The eluted product was amplified by 10 rounds of LM-PCR.

Illumina platform sequencing

a. Sequencing was performed via the standardized sequencing workflow of the Illumina HiSeq 2500 platform.

b. The raw data were obtained by analyzing the original sequencing data using the official BclToFastq software from Illumina.

c. Data analysis

• Yield analysis of raw data: Adapter contamination and low-quality data were removed.

• Alignment: The data obtained in step i were aligned to the reference sequence (the Burrows–Wheeler Alignment tool, BWA, was used for the alignment) using the hg19 genome as the reference genome.

• Single nucleotide polymorphism (SNP) detection and annotation: The results were analyzed using the SAMtools software.

• Insertion–deletion (Indel) detection and annotation: The Pindel software was used to analyze the results.

• False-positive filtering: According to the quality of the mutation analysis and the sequencing depth, the detected SNPs and Indels were filtered and screened to obtain high-quality and reliable mutations.

• Mutation annotation: Based on the locations of the SNPs and Indels in the gene, the effects on amino acids, splicing, untranslated regions, and intron mutations were determined.

• Prediction of the influence of the selected mutations on protein function: We used algorithms based on the homologous alignment and conservation of the protein structure and applied the scale invariant feature transform (SIFT) algorithm to predict the influence of the selected mutation on the protein.

• Splicing alternation predictions for mutations near the splice site.

2.2.3. Sanger sequencing verification. Primers were designed based on the sequences of the TSC sites for verification, and PCR amplification was performed. The PCR reaction conditions were as follows: pre-denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds; and an additional extension step at 72°C for 10 minutes to ensure that the PCR reaction was complete and to increase the amplification yield. The PCR system volume was 50 μL, and the primer sequences are presented in Table 1. For the gene sequence analysis, PCR amplification products of the TSC sites for verification were sequenced using the ABI 3730XL sequencer with the original PCR primers as the sequencing primers. The gene sequence analysis was performed using the DNASTAR software for sequence analysis and alignment.

3. Results

The targeted TSC genes in the probands were sequenced, and multiplex ligation-dependent probe amplification (MLPA) was used to detect deletions or duplications. Candidate sites were screened by database searching and bioinformatics analysis. For known point mutations, cosegregation analysis within the family was conducted via Sanger sequencing. For novel mutation sites, cosegregation analysis was conducted within the family, and the sites were verified in normal individuals to eliminate the possibility of polymorphisms. Our results showed that the

| Table 1 |
|---|
| Primer sequences to verify the sites in the TSC genes. |
| Family | Primer name | Base sequence | Annealing temperature, °C | Amplification product size, bp | mRNA alignment template |
|---|---|---|---|---|---|
| 1 | GN541-8F-1 | TCTCATGCGGTGGGTGTAGC | 60 | 1360 | TSC_NM_000548c.1228_c.1229insGp.L410Rfs*11 |
| | GN541-8R-2 | CTCTGCGGGAGCACGTGACAAC | 60 | 1278 | NM_000548 |
| 2 | F | CTCCGGCGAGTCAGTACGTGCCC | 60 | 1360 | TSC_NM_000548c.1228_c.1229insGp.L410Rfs*11 |
| | R | ACCAAGTACCTGGCCAGTACTCC |
Figure 3. Results of next-generation sequencing of the proband in family 1 (the long box marks the mutation site).

Figure 4. Sequencing results of the c.1228–1229 insG heterozygous mutation in the TSC2 gene c.1228 (exon 12)_c.1229 (exon 12) insG in the proband of Family 1 (the arrow indicates the mutation site).
Figure 5. Results of next-generation sequencing of the proband in Family 2 (the long box marks the mutation site).

Figure 6. Sequencing results of the c.4925 G>A heterozygous mutation in the TSC2 gene c.4925G>A (exon 38) in the proband of Family 2 (the arrow indicates the mutation site).
and the presence of unidentifiable gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]

The TSC1-encoded Hamartin and TSC2-encoded tuberin proteins have high affinity and form a heterodimer in the cytoplasm. Mutations in the TSC1 or TSC2 genes affect the function of the TSC1–TSC2 complex (also known as the hamartin–tuberin complex), resulting in an abnormality in the GAP domain (GTPase activator protein) of TSC2. In turn, this abnormality leads to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] Abnormalities in the GAP domain (GTPase activator protein) of TSC2 lead to the abnormal activation of the mammalian target of rapamycin (mTOR) signal transduction pathway and subsequently disease.[9] The detection of abnormalities in the GAP domain (GTPase activator protein) of TSC2 has been reported, covering almost all exons of the TSC1 and TSC2 genes. TSC2 mutations account for approximately 60% of TSC mutations, and TSC1 mutations account for approximately 30%.[1,6] However, no mutations have been found in 10% to 25% of all clinically diagnosed TSC patients.[13] Possible causes include insufficient detection sensitivity, mutations in introns or promoter regions, low-abundance somatic cell chimeric mutants in TSCs, and the presence of unidentified gene loci, such as the TSC3 gene.[8]
difficult to treat in many patients, and the mechanism underlying the
epilepsy is not clear. In most TSC patients, a correlation is
usually found between the location of the nodule and the affected
area, with the nodule considered to be an epileptogenic
cape. Because multiple nodules can be present, TSC patients may
develop multifocal or generalized seizure syndromes, such as
infantile spasms or Lennox–Gastaut syndrome. Repeated
seizures in patients with TSC may be caused by mutations in the
TSC1/TSC2 genes, abnormal cortical cell structures, changes in
synaptic connections, changes in the expression of neurotrans-
mittor receptors or ion channels, abnormal expression of growth
factors, and excessive proinflammatory responses. Thus, the
surgical removal of nodules should, logically, reduce seizures.
However, recent studies that placed a detection electrode on the
nodules and the surrounding cortex found no abnormal
discharge in the nodular region, whereas the surrounding cortical
tissue showed significant epileptic activity. This finding suggests
that the epileptogenic lesion cannot be in the nodule itself but
may instead be in the cortex around the nodule[22] and may cause
uncontrolled seizure symptoms in some patients after nodule
resection.

In summary, children’s TSC most often affects the nervous
system, which not only leads to severe abnormalities in mental
and motor development but also often causes death. This disease
causes serious pain to the child, burdens to family and society.
Therefore, prenatal examination is particularly important. The 2
new mutations detected in this study were presumed to be
pathogenic mutations, which enriched the spectrum of gene
mutations. However, genetic mutations eventually lead to the
pathological process of injury, which is under the effects of
various factors, including the internal and external environments.
The pathogenesis is very complex and remains unclear; thus, the
functions of the mutated proteins need to be validated using cell
and animal models. The discovery of new mutations not only
provides a basis for prenatal genetic diagnosis for the affected
family but also more research entry points.

Author contributions
Conceptualization: Shan Gao, Yongmei Xie.
Data curation: Shan Gao, Yongmei Xie.
Formal analysis: Shan Gao.
Funding acquisition: Zhiling Wang.
Investigation: Shan Gao.
Methodology: Shan Gao.
Writing – original draft: Shan Gao.
Writing – review & editing: Zhiling Wang.

References
[1] Ismail NF, Nik Abdul Malik NM, Mohseni J, et al. Two novel gross
deletions of TSC2 in Malaysian patients with tuberous sclerosis complex
and TSC2/PKD1 contiguous deletion syndrome. Jpn J Clin Oncol
2014;44:506–11.
[2] Orlova KA, Crino PB. The tuberous sclerosis complex. Ann NY Acad Sci
2010;1184:87–105.
[3] Osborne JP, Fryer A, Webb D. Epidemiology of tuberous sclerosis. Ann
NY Acad Sci 1991;615:125–7.
[4] Huang CH, Peng SS, Weng WC, et al. The relationship of neuroimaging
findings and neuropsychiatric comorbidities in children with tuberous
sclerosis complex. J Formos Med Assoc 2015;114:849–54.
[5] Jones AC, Daniels CE, Snell RG, et al. Molecular genetic and phenotypic
analysis reveals differences between TSC1 and TSC2 associated familial
and sporadic tuberous sclerosis. Hum Mol Genet 1997;6:2155–61.
[6] Jones AC, Shyamsundar MM, Thomas MW, et al. Comprehensive
mutation analysis of TSC1 and TSC2 and phenotypic correlations in
150 families with tuberous sclerosis. Am J Hum Genet 1999;64:
1305–15.
[7] Krueger DA, Northrup H. International Tuberous Sclerosis Complex
Consensus Group Tuberous sclerosis complex surveillance and manage-
ment: recommendations of the 2012 International Tuberous Sclerosis
Complex Consensus Conference. Pediatr Neurol 2013;49:255–65.
[8] Qin W, Kozlowski P, Tailion BE, et al. Ultra deep sequencing detects a
low rate of mosaic mutations in tuberous sclerosis complex. Hum Genet
2010;127:573–82.
[9] Astrandis A, Henske EP. Tuberous sclerosis complex: linking growth and
energy signaling pathways with human disease. Oncogene 2005;24:7475–81.
[10] Huang J, Manning BD. The TSC1-TSC2 complex: a molecular
switchboard controlling cell growth. Biochem J 2008;412:179–90.
[11] De Waal L, Lagae L, Meckali D. Tuberous sclerosis complex: the past
and the future. Pediatr Nephrol 2015;30:1771–80.
[12] Mazhab-Jafari MT, Marshall CB, Ho J, et al. Structure-guided mutation
of the conserved G3-box glycin in Rheb generates a constitutively
activated regulator of mammalian target of rapamycin (mTOR). J Biol
Chem 2014;289:12195–201.
[13] Mayer K, Goedbloed M, Van Zijl K, et al. Characterisation of a novel
TSC2 missense mutation in the GAP related domain associated with
minimal clinical manifestations of tuberous sclerosis. J Med Genet
2004;41:e64.
[14] Yu Z, Zhang X, Guo H, et al. A novel TSC2 mutation in a Chinese family
with tuberous sclerosis complex. J Genet 2014;93:169–72.
[15] Pan YC, Wu WQ, Xie JS, et al. Two novel TSC2 frameshift mutations in
tuberous sclerosis complex. Zhongguo Dang Dai Er Ke Za Zhi
2001;3:64.
[16] De Waele L, Lagae L, Mekahli D. Tuberous sclerosis complex: the past
and the future. Pediatr Nephrol 2015;30:1771–80.
[17] Dabrowska E, Zuziak W, Tafert CT, et al. Structural and mRNA
expression analysis of TSC1 and TSC2 in patients with tuberous
sclerosis complex. J Formos Med Assoc 2015;114:849–54.
[18] Maldonado M, Baybis M, Newman D, et al. Expression of ICAM-1,
TNF-alpha, NF-kappa B, and MAP kinase in tubers of the tuberous
sclerosis complex. Zhongguo Dang Dai Er Ke Za Zhi
2001;3:64.
[19] Orlova KA, Crino PB. The tuberous sclerosis complex. Ann NY Acad Sci
1991;615:125–7.
[20] Boer K, Crino PB, Gorter JA, et al. Gene expression analysis of tuberous
sclerosis complex cortical tubers reveals increased expression of adhesion
and inflammatory factors. Brain Pathol 2010;20:704–19.
[21] Crino PB. Evolving neurobiology of tuberous sclerosis complex. Acta
Neuropathol 2013;125:317–32.