

# Early diagnosis of a newborn with tuberous sclerosis caused by a genetic mutation

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## Abstract

**Objective:** Tuberous sclerosis (TSC) is an autosomal dominant disorder, often detected during childhood. We present the results of genetic testing in a newborn with suspected TSC.

**Methods:** A newborn with no specific clinical manifestations of TSC showed evidence of TSC on magnetic resonance imaging and echocardiography. Next-generation sequencing (NGS) and multiple ligation-dependent probe amplification (MLPA) of the *TSC1* and *TSC2* gene exons were carried out to confirm the diagnosis.

**Results:** The results of MLPA were negative, but NGS showed a heterozygous mutation in the *TSC1* gene comprising insertion of a T residue at c.2165 (exon 17) to c.2166 (exon 17), indicating a loss of function mutation. These results were verified by Sanger sequencing. This genetic change was present in the newborn but the parental genotypes were wild-type, indicating a *de novo* mutation.

**Conclusions:** In this case, a case of TSC caused by a heterozygous mutation in the *TSC1* gene was confirmed by NGS sequencing. This indicates the suitability of genetic testing for the early diagnosis of clinically rare and difficult-to-diagnose diseases, to guide clinical treatment.

## Keywords

Gene, intensive care unit, neonate, tuberous sclerosis, next-generation sequencing, *TSC1*

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## Introduction

Tuberous sclerosis (TSC) is an autosomal dominant disorder with an incidence of about 1 in 6000 individuals. It occurs worldwide, with no ethnic or sex differences.<sup>1</sup> About 33% of patients have a family

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history of TSC while the remaining 67% are sporadic cases.<sup>2</sup> TSC often occurs during childhood. It is characterized by clinical manifestations involving the heart, brain, kidney, skin, and other organs, leading to abnormal dysplasia and hamartomatous changes.<sup>3</sup> However, the clinical manifestations of TSC are not consistent among patients, making it extremely difficult to identify. TSC is caused by mutations in the *TSC1* or *TSC2* gene,<sup>4</sup> and the TSC Diagnostic Consensus Standard (2012) emphasizes that TSC can be diagnosed definitively by identifying pathogenic mutations in these genes in DNA from normal tissue, regardless of the clinical characteristics.<sup>3</sup> Nevertheless, the relatively large sizes of these genes means that no hot spots or common mutations have yet been identified.<sup>5</sup> Furthermore, even gene mutations at the same locus can lead to different clinical manifestations.<sup>5</sup> However, the combination of different genomic detection methods greatly improves the detection rate of *TSC1* or *TSC2* mutations. There are few reports of neonates with TSC because of its insidious signs and symptoms in the neonatal period. We report the case of a newborn with no obvious TSC features who was suspected to have TSC based on echocardiography and head magnetic resonance imaging (MRI). The diagnosis was confirmed by multiple ligation-dependent probe amplification (MLPA) and next-generation sequencing (NGS).

## Materials and methods

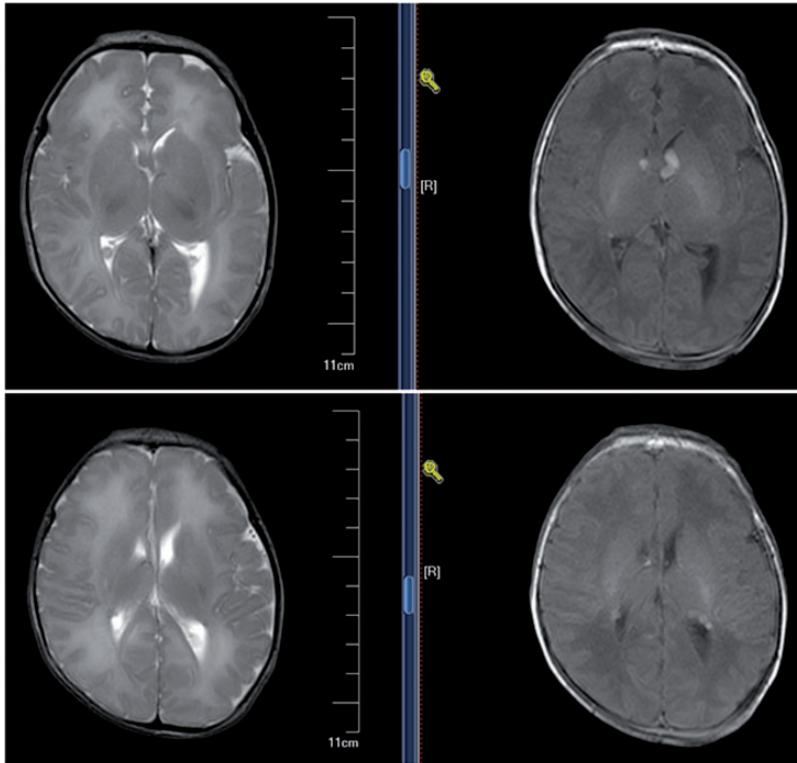
### Patient

The newborn was referred to our clinic with dyspnea, sepsis, and hypoglycemia on 10 December 2018. The male child was born at 38<sup>+1</sup> weeks via cesarean section because of a 3-hour-premature rupture of membranes. The infant's birth weight was 2740 g. The clinical manifestations of the

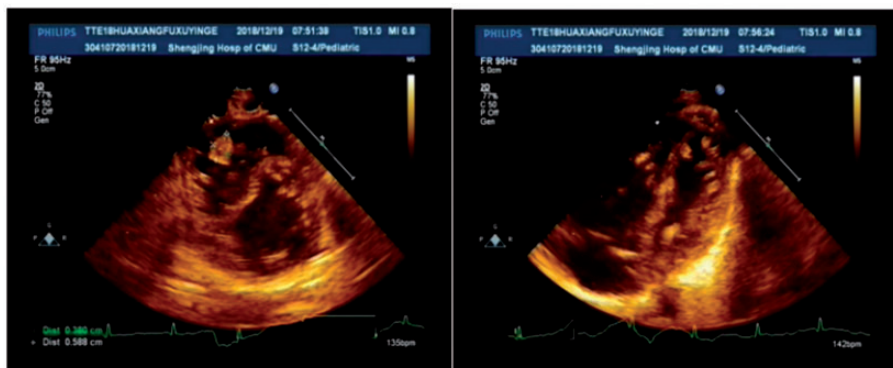
newborn were dyspnea, spitting, cyanosis of the lips, no signs of nasal fan, and supra-sternal, intercostal, and subcostal retractions indicative of dyspnea. These early manifestations did not support a diagnosis of TSC. The amniotic fluid had third-degree contamination, but no abnormalities were found in the umbilical cord or placenta. A family history of inherited metabolic diseases was denied. This case report was approved by the Ethics Committee of Shengjing Hospital of China Medical University (No. 2020ps680k). Written informed consent for the procedures and for publication was obtained from the patient's father.

After birth, the infant's dyspnea was treated with oxygen inhalation at low flow with an inhaled oxygen concentration of 28% for 1 day. The highest C-reactive protein level after birth was 31.4 mg/L, and ceftriaxone (20 mg/kg, every 12 hours) was given to treat infections. The lowest blood glucose level was 2.2 mmol/L, and the newborn was given intravenous glucose (6 mg/kg/minute). The blood platelet count at birth was  $81 \times 10^9$  but fell to  $43 \times 10^9$  at 3 days after birth, after which gamma globulin was given for 3 days and the platelet count returned to  $136 \times 10^9$ . The newborn had no history of convulsions after birth.

When the patient developed sepsis, we performed head MRI and cardiac ultrasound examinations. Head MRI showed visible white spots and multiple small fragments of short longitudinal relaxation time (T1) signals under the ependyma of the bilateral ventricles (Figure 1). Color Doppler echocardiography indicated strong echoes from multiple nodular hyper-echoic lesions in the left and right chambers of the heart and the left ventricular myocardium. Rhabdomyosarcoma was noted, and ventricular non-compaction cardiomyopathy was observed in both ventricles (Figure 2). These results suggested the possibility of TSC. Renal color Doppler



**Figure 1.** Magnetic resonance imaging results of the newborn's brain showing visible white spots and multiple small fragments of short longitudinal relaxation time (T1) signals under the ependyma of the bilateral ventricles.



**Figure 2.** Color Doppler echocardiography showed strong echoes from multiple nodular hyperechoic lesions in the left and right chambers of the heart and the left ventricular myocardium. Rhabdomyosarcoma was noted, and ventricular non-compaction cardiomyopathy was observed in both ventricles.

ultrasound of both kidneys revealed no abnormalities. Ambulatory electroencephalography, dynamic electrocardiography, and fundus examination were refused. Considering the possibility of a clinical diagnosis of TSC, NGS and DNA sequence analysis were suggested to confirm the diagnosis.

### **Specimen collection**

After obtaining informed consent, blood was collected from the newborn and anticoagulated with EDTA. Whole-blood DNA was extracted using a Blood Genomic DNA Midi Kit (CW BIO, Beijing, China), in accordance with the kit instructions. The quality of the extracted DNA was determined using a Qubit 2.0 fluorometer (Thermo Electron Co., Waltham, MA, USA) and 0.8% agarose gel electrophoresis.

### **NGS**

The xGen<sup>®</sup> Exome Research Panel v1.0 capture probe (Illumina, San Diego, CA, USA) and genomic DNA library sequence were used to perform liquid hybridization and to concentrate the DNA fragments in the target region to construct a whole-exome library covering the coding regions of 19,396 genes and part of the non-coding regions, with a capture interval of 51 Mb. The sequenced genome in the target sequence achieved coverage of >99% using a high-throughput sequencer (Illumina NovaSeq 6000 series, Illumina Connected Analytics, Integrated DNA Technologies, Collerville, IA, USA).

### **DNA sequence analysis**

Quality control was conducted on the original data to remove joint and low-quality reads. High-quality, reliable mutations were detected by comparing the sequence with the reference genomes GRCh37/hg19

of Ensembl using the Burrows–Wheeler Aligner. The Genome Analysis Toolkit was applied to analyze single-nucleotide polymorphisms (SNPs) and indels, and the SNPs and indels were filtered and sifted according to the sequencing depth and the quality of the mutations.

High-quality variations were annotated by scanning large databases, including the dbSNP, 1000-Genomes, ExAC, and other genome-aggregation databases, OMIM, HGMD, and ClinVar, using self-developed variation-annotation software. The criticality of the variations was analyzed using Proven, SIFT, Polyphen2-HVAR, Polyphen2-HDIV, M-Cap, Revel, Mutation taster, and MaxEntScan, to screen out variations with a harmful effect on the protein structure.

### **MLPA**

We used MLPA to screen the patient for large copy number changes. We scanned the *TSC1* and *TSC2* genes using the SALSA MLPA probe mix P124-C1 and P046-C1 kits (MRC-Holland; Amsterdam, the Netherlands), respectively, in accordance with the kit instructions. After collecting blood and extracting the DNA from the newborn as described above, the DNA was denatured at 98°C for 5 minutes, and then incubated with hybridization master mix (1.5 µL SALSA probe mix + 1.5 µL MLPA buffer) at 95°C for 1 minute and hybridized at 60°C for 16 hours. The temperature was adjusted to 54°C, and 32 µL of ligase-65 master mix solution (3 µL ligase buffer A + 3 µL ligase buffer B + 25 µL ultrapure water + 1 µL ligase-65) was added and incubated at 54°C for 15 minutes. The polymerase mix solution (7.5 µL ultrapure water + 2 µL polymerase chain reaction (PCR) primer mix + 0.5 µL SALSA polymerase) was added at room temperature. The PCR program included 35 cycles of 95°C for 30 s,

60°C for 30 s, and 72°C for 60 s, followed by 72°C for 20 minutes, pausing when the temperature dropped to 15°C. Fragment analysis was performed by capillary electrophoresis using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Coffalyser was used to analyze the copy number changes in the *TSC1* and *TSC2* genes.

### Verification by Sanger sequencing

Sanger sequencing was used to confirm the mutation. The primers were designed based on the sequences of the *TSC* sites for verification, and PCR amplification was performed for 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and extended at 72°C for 10 minutes to ensure completion of the PCR reaction and increase the amplification yield. The PCR product was sequenced using an ABI PRISM 3100 genetic analyzer, analyzed using DNASTAR software, and compared with the mRNA template (*TSC1*: NM\_000368).

## Results

### Genetic testing

MLPA showed no abnormalities in the *TSC1* and *TSC2* gene exon copy numbers, and no exon deletions or duplications of the *TSC1* and *TSC2* genes were detected (Figure 3). High-throughput sequencing of the whole exons revealed a heterozygous mutation in the *TSC1* gene, involving the insertion of a thymine residue at c.2165 (exon17) to c.2166 (exon17). The test results were verified by Sanger sequencing, which showed a standard sequence of GGCGGCTCCTCCGCAAGTGATCAAAGCAGCA, and a pathogenic sequence of GGCGGCTCCTCCGCAATGTGATCAAAGCAGCA. Genetic sequencing of the parents showed that both had wild-type

genes, indicating that the genetic change in the newborn was a *de novo* mutation (Figure 4). The insertion of a T at nucleic acid position c.2165 to c.2166 in exon 17 of NM\_000368 transcript results in an amino acid substitution from lysine to asparagine at position 722, leading to a mutant sequence containing 12 substituted amino acid residues encoded by the insert followed by a stop codon that truncates the protein. The patient was diagnosed with TSC, confirmed by genetic examination.

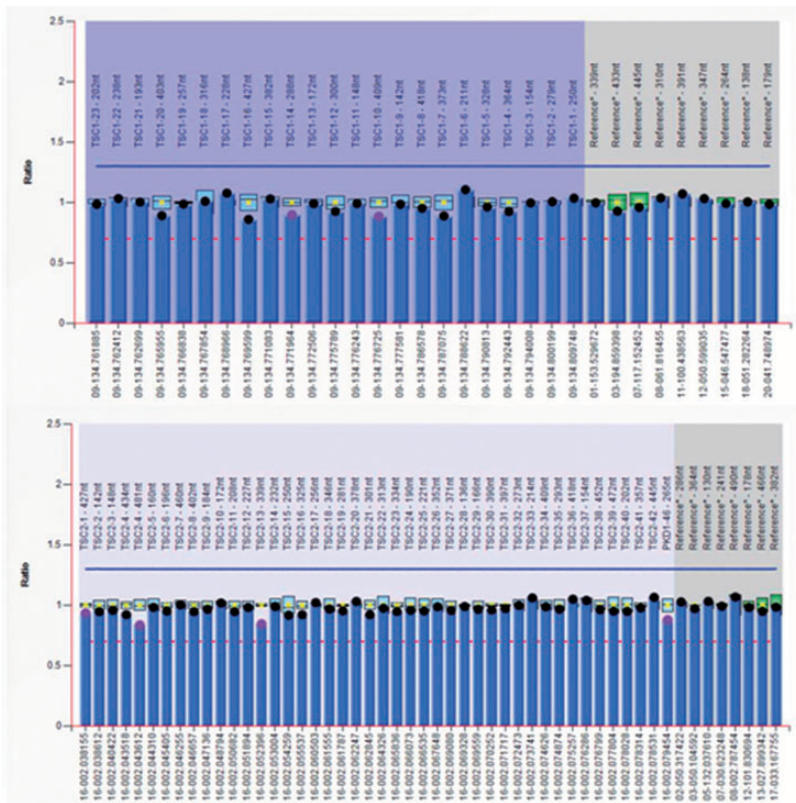
### Treatment

The newborn was given a continuous intravenous infusion of gamma globulin (1 g/kg/day) for 3 days to block antibodies and neutralize toxins, intravenous creatine phosphate (0.5 g/day) for 1 week to support the myocardium, and ceftriaxone sodium to fight infection (20 mg/kg, every 12 hours).

After 12 days of hospitalization, the infant showed satisfactory feeding tolerance, stable weight gain, physiological blood glucose levels, appropriate reflexes, and a stable body temperature. The family members requested that the infant be discharged. During the 6-month follow-up period, the child had no other findings related to TSC, and did not receive rapamycin. At the time of completion of this manuscript, the patient was 2.5 years old, had no symptoms such as convulsions, showed good growth and development, and had a lively personality.

## Discussion

TSC is mainly caused by mutations in the *TSC1* and *TSC2* genes. The *TSC1* gene contains 23 exons and is 53 kb long. *TSC1* mutations lead to type 1 TSC. The *TSC2* gene contains 42 exons and is 40.7 kb long. Seventy-five percent of patients with TSC have mutations in the *TSC2* gene, mostly *de novo* mutations, leading to type 2

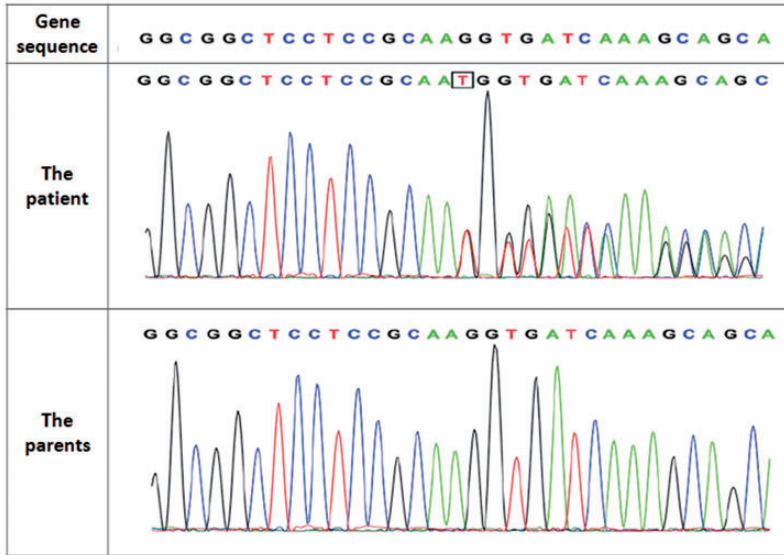


**Figure 3.** No exon deletions or duplications of the *TSC1* and *TSC2* genes were detected by multiplex ligation-dependent probe amplification. The normal ratio ranges from 0.7 to 1.3, with values  $>1.3$  indicating suspected duplication and  $<0.7$  suspected missing.

*TSC*.<sup>6</sup> The *TSC1/TSC2* complex is a key inhibitory signal integrator within cells that regulates mammalian target of rapamycin (mTOR) signaling pathways to control the physiological processes involved in cell growth, metabolism, proliferation, and apoptosis.<sup>7</sup> Changes in the *TSC1/TSC2* complex modulate mTOR signaling, leading to abnormal dysplasia in some organs and hamartomatous changes.

Bourneville discovered TSC in 1880 and was the first to describe its neurological symptoms and pathological changes. Heinrich Vogt then described the triad of the disease, namely epilepsy, mental retardation, and facial angiofibroma, in 1908.<sup>8</sup> The first manifestations of this disease are

often neurological, such as cognitive impairment, epilepsy, mental retardation, and autism.<sup>9,10</sup> The disease fundamentally involves a series of clinical manifestations reflecting the invasion of multiple organs, such as the skin, nerves, heart, and kidneys. The TSC diagnostic criteria developed by the International Tuberous Sclerosis Complex Consensus Conference in 2012<sup>3</sup> depend on the following major clinical manifestations: (1) facial angiofibromas ( $\geq 3$ ) or fibrous cephalic plaques; (2) ungual fibromas; (3) hypomelanotic macules; (4) shagreen patch; (5) multiple retinal nodular hamartomas; (6) cortical dysplasias, including tubers and cerebral white matter radial migration lines;



**Figure 4.** *TSC1* gene sequences in the patient and his parents according to high-throughput sequencing.

(7) subependymal nodules; (8) subependymal giant cell astrocytoma; (9) cardiac rhabdomyomas (single or multiple); (10) pulmonary lymphangioliomyomatosis; and (11) renal angiomyolipomas. Minor features include (1) dental enamel pits ( $\geq 3$ ); (2) intraoral fibromas ( $\geq 2$ ); (3) non-renal hamartomas; (4) retinal achromatic patch; (5) mottled skin lesions; and (6) multiple renal cysts.

A definite diagnosis of TSC is confirmed when a patient has two major features or one major feature with at least two minor features, and a possible diagnosis of TSC is made when patients have either one major feature, one major feature with one minor feature, or only minor features. A definitive diagnosis can also be made on the basis of a pathogenic mutation in the *TSC1* or *TSC2* gene detected by genetic testing. The present case had two major features of TSC (subependymal nodules and multiple cardiac rhabdomyomas), which might be sufficient to make a definitive clinical diagnosis of TSC.

Genetic testing in the present newborn indicated a *TSC1* gene alteration, which confirmed the diagnosis of TSC. About 10% of patients with TSC have copy number variations in the *TSC1* or *TSC2* gene, and although some patients with TSC have no detected genetic variations, the possibility of heterozygosity cannot be excluded. In this case study, no deletions or duplications of TSC gene exons were detected by MLPA, but *TSC1* heterozygosity was found by high-throughput detection. Insertion of a thymine had occurred in one DNA strand of exon 17 of *TSC1*, resulting in a frameshift mutation that led to a change in the amino acid of p. K722Nfs\*12, indicating a loss of function mutation. This genetic variation has not previously been reported in the literature, and its minor allele frequency is  $<0.005$ , indicating a low frequency. In addition, this genetic change was only present in the newborn patient and not in the parents, indicating that it was a *de novo* mutation. This variation might be classified as a

pathogenic mutation according to the American College of Medical Genetics and Genomics guidelines (2015)<sup>11</sup> because its pathogenic criterion was weighted as PVS1 + PS2 + PM2. PVS1 indicates very strong pathogenic evidence, indicating a null variation that might result in the loss of gene function; PS2 indicates strong pathogenic evidence, in which a new mutation is confirmed by examining both parents; and PM2 indicates moderate pathogenic evidence with a low-frequency variation. The genetic change in the current newborn patient was not accompanied by clinical symptoms, but imaging studies confirmed the presence of corresponding TSC-associated changes.

## Conclusion

TSC is an autosomal dominant disorder characterized by symptoms involving hamartoma invasion of multiple organs. In this study, TSC-associated changes were revealed by head MRI and echocardiography of the newborn, but no noticeable clinical symptoms were observed, suggesting that some patients might develop TSC in the neonatal period but might not be diagnosed because of the absence of noticeable symptoms. Further clinical follow-up, including monitoring of the nervous system and other organs, will be required to determine if the present patient develops other clinical manifestations in the future. It was recommended that the newborn should be followed-up regularly, and oral rapamycin should be administered if necessary. The infant's growth and development were good at 6 months, with a weight of 8.5 kg and no convulsions or other clinical symptoms.


## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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