Abstract. Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease that varies greatly in its expression. The current study reports a novel case of TSC caused by a TSC2 mutation (TSC2c.1642_1643insA or TSC2p. K549fsX589), in which multiple cardiac rhabdomyomas were detected by fetal echocardiography in week 31 of pregnancy. The infant was delivered successfully; however, seizures began 16 days following birth. Subsequent genetic tests confirmed a diagnosis of TSC. Rapamycin treatment resulted in regression of cardiac rhabdomyomas and controlled seizures. The current study demonstrates the value of fetal echocardiography in the diagnosis of TSC and suggests that inhibition of the mammalian target of the rapamycin (mTOR) signaling pathway may be considered as a potential antiepileptogenic therapy for neonatal TSC. In addition, it was demonstrated that rapamycin treatment was therapeutically beneficial for preventing disorders caused by abnormal mTOR signaling, such as cancer. According to the literature, cardiac rhabdomyomas, seizures and skin lesions are well established markers for TSC in neonates. MRI scans of the brain and genetic screening of TSC1 and TSC2 genes may facilitate an early diagnosis of TSC.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease with an incidence of 1 in 6,000 and its symptoms include seizures, mental retardation, skin lesions and the formation of hamartomas in multiple organs, including the heart, brain, eye and kidney (1). Mutations in one of the two tumor suppressor genes TSC1-9q34 and TSC2-16p13.3 are responsible for TSC (2). The absence of clinical features during the neonatal period makes the diagnosis of TSC difficult. Neonatal ultrasound and cerebral magnetic resonance imaging (MRI) may be used to detect hamartomas in the heart and brain (3). Additionally, genetic mutation analysis means that the risk of a couple conceiving a child with TSC can be determined prior to pregnancy (4). Shepherd et al (5) analyzed 355 cases of TSC and reported that the mortality rate is 13.8%.

Medication and surgery are the major treatment methods for TSC (6,7). Vigabatrin, an antiepileptic medicine, was approved in 2009 and recommended as a first-line drug for TSC-associated infantile spasms by 2012 (8). In addition, adrenocorticotropic hormone was approved to treat infantile spasms in 2010 (9). Everolimus is applied for brain (subependymal giant cell astrocytoma) and kidney (renal angiomyolipoma) tumor treatment in children with TSC (10) and has also been demonstrated to be effective for TSC-epilepsy treatment (11). In 2017, vorstuba was recommended by the European Commission as a treatment for refractory partial-onset seizures in patients with TSC (12). Canpolat et al (13) demonstrated that rapamycin effectively controls epilepsy without causing any marked side effects in children with TSC.

The current study describes a case of multiple cardiac rhabdomyomas confirmed by routine echocardiogram screening in week 31 of pregnancy. The infant experienced seizures in the neonatal period (2 weeks of age), which is earlier than previously reported cases in which the onset was 1 month of age (14). Genetic mutation analysis revealed a novel mutation in TSC2. The clinical presentation and final outcome of neonatal TSC is discussed in the current study.

Case report

The mother of the infant in the current case report was a 29-year-old female (gravida 2, para 0) who experienced an uncomplicated pregnancy until week 31 of gestation. A routine echocardiography examination revealed multiple substantial hyperechoic masses in the ventricular wall of the fetus, suggesting a diagnosis of cardiac rhabdomyomas. A male infant weighing 3 kg was successfully delivered in week 39 of pregnancy. At 23 days old, the infant was admitted to the Emergency Department of the Children’s Hospital, Zhejiang University School of Medicine (Hangzhou, China) in January 2016. The infant had undergone a series of active seizures in the week prior to admittance. Seizure episodes
were characterized by paroxysmal jittery limbs followed by the passing of urine. Each episode lasted 5-10 sec and was not accompanied by fever. The parents of the infant were healthy and had no history of TSC. The patient’s family denied a history of epilepsy, mental retardation and behavioral problems.

Physical examination revealed a small number of hypomelanotic macules on the skin on the chest and back of the infant. The results of the neurological (including physiological reflex) and cardiovascular (heart rate, 124 bpm; blood pressure, 74/40 mmHg) examinations were normal. MRI scans of the brain identified subependymal nodules and subcortical tubers (Fig. 1), which are two distinct features of TSC. Echocardiography revealed hyperechoic masses with clear borders and uniform echoes, measuring 1.06x0.89 and 1.77x1.68 cm in the left atrium and the outlet of the right ventricle, respectively, verifying the presence of intracardial tumors, which indicated the presence of cardiac rhabdomyomas (Fig. 2). Ophthalmological examination clearly identified multiple nodules (Fig. 3). A detailed abdominal ultrasound did not identify any signs of TSC. Electrocardiogram revealed a sinus rhythm without cardiac arrhythmia and video electroencephalographic monitoring did not prompt a diagnosis of epileptic seizures. Mutation analysis of the TSC1 and TSC2 genes confirmed a diagnosis of TSC. A TSC2c.1642_1643insA [or TSC2p.K549fsX589; TSC2 normal gene reference is from NP_0,01070651.1 (https://www.ncbi.nlm.nih.gov/protein/116256350/)] frameshift mutation was identified, which terminated the translation of the encoded protein (Fig. 4). No mutations were identified in the TSC1 sequence.

Following diagnosis of TSC, the patient received antiepileptic drugs including Topamax® (3 mg/kg/day; Xian-Janssen Pharmaceutical Ltd., Xi’an, China), Depakin® (30 mg/kg/day; Sanofi S.A., Paris, France) and nitrazepam (1 mg/kg/day; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). However, this treatment did not alter the frequency of seizures. At the age of 3 months, the infant underwent treatment with 1 mg/(m²/day) rapamycin orally to treat resistant epileptic seizures and the blood concentration of rapamycin was maintained at 5-10 µg/l, as measured using the Rapamycin ARCHITECT Sirolimus Reagent kit (cat. no. 72003M800) and the Abbott Architect i1000 (both Abbott Pharmaceutical Co.,

Figure 1. Neonatal (23-day-old) brain MRI. (A) Axial plane and (B) sagittal plane MRI images of a neonate with tuberous sclerosis complex at the first visit. The subependymal nodule is indicated by an arrow. MRI, magnetic resonance imaging.

Figure 2. Echocardiographic images of cardiac rhabdomyomas located in (A) the LA, measuring 1.06x0.89 cm and (B) the outlet of the RV, measuring 1.77x1.68 cm. Arrow indicates the cardiac rhabomyomas. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; FR, frame rate; 2D, two dimensional; dist, distance.

Figure 3. Ophthalmological examination revealing multiple clearly visible nodules.
During this period of 3 months, seizures and results of an electroencephalogram (EEG) were recorded every month and the results of the routine blood and urine tests, and liver and kidney function tests (including glutamic-pyruvic transaminase, normal range at 8-40 U/l and serum creatinine, normal range at 15-77 µmol/l) were normal.

Echocardiography performed on the infant at the age of 6 months identified regression of multiple cardiac rhabdomyomas and MRI of the brain revealed a decrease in the size of cerebral lesions (Fig. 5). The results of the EEG were normal and the frequency of seizures decreased. However, the patient continued to exhibit mental retardation.

Discussion

A thorough search of literature published since 1990 (384 published manuscripts, including 98 reviews) was performed using PubMed (https://www.ncbi.nlm.nih.gov/pubmed) with the following key words: Tuberous sclerosis complex, newborn, neonates, neonatal, infants. Data from 36 infants aged <4 weeks who were diagnosed with TSC were included in the review. The clinical manifestations of TSC were present prior to birth in 8 patients (22.22%), at birth in 13 patients (36.11%) and by 4 weeks of age in 7 patients (19.44%). Information regarding onset of TSC was not available for the remaining 8 patients included in the current review (22.22%). TSC was diagnosed at birth in 7 patients (19.44%) and by 4 weeks of age in 29 patients (80.56%). A greater number of males than females (ratio, 1.4:1) were diagnosed with TSC. There was a family history of TSC in 2 patients (5.55%). The most common features at onset of TSC were cardiac rhabdomyomas (36.11%), seizures (19.44%), arrhythmia (16.67%) and skin lesions (13.89%) followed by renal cyst, opisthotonus, feeding difficulties and respiratory distress. A total of 34 neonates underwent brain MRI or computed tomography imaging and 2 neonates underwent a brain biopsy following mortality. Cortical tubules, subependymal nodules and subependymal giant cell astrocytomas were identified in 26 (72.22%), 28 (77.78%) and 5 (13.89%) patients, respectively. Retinal hamartomas were detected in 5 neonates. The overall survival rate of neonates with TSC was 81% (21/26) and the duration of follow-up varied from 1 month to 4 years.

Cardiac rhabdomyomas were the most common initial symptom detected in neonates with TSC included in the current review and were identified in 13 neonates (36.11%). Cardiac rhabdomyomas are the most prevalent heart tumors (15). It has been reported that >80% of cardiac rhabdomyomas regress completely during infancy and early childhood (16). Depending on their size, location and number, cardiac rhabdomyomas cause serious cardiovascular complications, including intracavitary obstruction, diminished myocardial function and arrhythmia (17). Medical and/or surgical interventions are required for symptomatic patients with hemodynamically significant cardiac rhabdomyomas or a life-threatening arrhythmia (18). In the present review, 2 out of 7 neonates with arrhythmia succumbed following cardiac arrest but the other 5 neonates survived following effective antiarrhythmic treatment.

Neurological manifestations including seizures and mental retardation are the major factors for morbidity in patients with TSC (19). The current review indicated that ~20% of neonates with TSC develop seizures in the first month of life. Early onset of epilepsy in TSC is strongly associated with mental retardation (20,21). It has been demonstrated that antiepileptic treatment reduces the severity of epilepsy and risk of mental retardation in infants with TSC (22,23). Brain lesions in TSC include cortical tubers, subependymal nodules (SEN), subependymal giant cell astrocytomas and white matter lesions (24). In the current review, cortical tubers and SEN were the two most common brain MRI manifestations in neonates with TSC.
Cutaneous manifestations of TSC are easily identified and are present in >90% of patients with TSC (25). Hypomelanotic macules may be present at birth, however they also may not appear until later in life (26). The ocular symptoms of TSC are retinal hamartomas and these occur in 40-50% of patients. Retinal hamartomas typically do not cause visual dysfunction (27) and ocular hamartomas rarely occur in neonates (28). However, in the current case, hypomelanotic macules and ocular hamartomas were present.

Renal lesions serve an important role in the course of TSC by impairing renal function (29). Renal cysts, angiomylipomas and renal cell carcinomas are the most common renal lesions in patients with TSC (30,31). Isacs (32) reported that 13.2% of neonates and fetuses with TSC exhibit renal cysts. Polycystin 1, transient receptor potential channel interacting (PKD1) is the major gene responsible for autosomal dominant polycystic kidney disease. The TSC2 gene lies adjacent to PKD1, suggesting that PKD1 serves a role in the etiology of renal cystic disease in TSC (33). Deletion of the TSC2 and PKD1 genes is associated with a severe polycystic phenotype and this occurs in 2% of patients with TSC (34).

TSC is caused by mutations in either of the two tumor suppressor genes, TSC1, which encodes hamartin (35) and TSC2, which encodes tuberin (36). TSC1 and TSC2 inhibit the mechanistic target of the mTOR-mediated signaling pathway, thus preventing cell growth and cell cycle progression (37). Dysfunction of TSC1/TSC2, which may be caused by a mutation, results in the loss of control of mTOR signaling and subsequently causes cancer. A novel TSC2 mutation was present in the current case report. The earlier diagnosis of patients with TSC2 mutations may be beneficial for reducing the severity of symptoms with earlier intervention (38). Rapamycin inhibits the activation of the mTOR signaling pathway and has been used to treat patients with TSC (39). Several studies have demonstrated successful regression of lesions in the skin, brain, and kidney (40-42). Canpolat et al (13) demonstrated that rapamycin effectively controls epilepsy without causing any marked side effects in children with TSC. However, to the best of our knowledge, there have been no studies in English investigating the effect of rapamycin on epilepsy in neonates and the safety of rapamycin in patients <18 years of age, particularly in infant and neonates, remains unknown (43). The patient in the current case study received rapamycin treatment and had a good prognosis, experiencing regression of cardiac rhabdomyomas and controlled seizures. Therefore, the current case report indicated that rapamycin treatment for TSC caused by a TSC2 mutation was therapeutically beneficial and may be beneficial in treating other disorders caused by abnormal mTOR signaling, such as cancer.

The current study demonstrates that cardiac rhabdomyomas, seizures and skin lesions are well established markers for TSC in neonates. MRI scans of the brain and genetic screening of TSC1 and TSC2 genes may facilitate an early diagnosis of TSC.

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