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Autopsy case of right ventricular rhabdomyoma in tuberous sclerosis complex

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Abstract

Tuberous sclerosis complex (TSC) is a genetic multisystem disorder characterized by widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung, and liver. Rhabdomyoma is the most common cardiac tumor diagnosed in fetuses, neonates and infants, and is closely linked to TSC. Here we describe an autopsy case of right ventricular rhabdomyoma in TSC. The deceased was a 3-month-old male infant, and TSC with a cardiac tumor had been diagnosed before his death. Since the cardiac tumor had not been physically blocking the blood flow, he had not undergone surgical intervention. At autopsy, the patient's height was 62 cm and his body weight was 6 kg. The heart weighed 37.3 g and the right ventricle was filled with the tumor. The tumor measured 2.1cm × 1.6cm, being a fusion of multiple tumors with several attachment sites to the myocardium. Histologically, the tumor was diagnosed as a rhabdomyoma, and was positive for mammalian target of rapamycin (mTOR). The brain weighed 795.0 g, without hydrocephalus. The cut surface of the brain revealed multiple cortical tubers and subependymal nodules. Through screening for the TSC1 (hamartin) and TSC2 (tuberin) genes, a nonsense mutation, c.1108C>T:p.Gln370*, was detected in the TSC2 gene. Immediate cause of death was determined to be ventricular obstruction by a cardiac rhabdomyoma with insidious growth. This case highlights the need for forensic pathologists to perform a complete autopsy to determine the cause of sudden death with cardiac tumor, including genetic examination.

Keywords: tuberous sclerosis complex, cardiac rhabdomyoma, obstruction, tuberin, forensic pathology, genetic analysis

1. Introduction

Tuberous sclerosis complex is a genetic multisystem disorder characterized by widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung, and liver [1]. Major clinical features in the 2012 International Tuberous Sclerosis Complex Diagnostic Criteria include hypomelanotic macules, angiofibromas, ungual fibromas, shagreen patch, retinal hamartomas, cortical dysplasias, subependymal nodules, subependymal giant cell astrocytoma (SEGA), cardiac rhabdomyoma, lymphangioleiomyomatosis and angiomyolipomas [2]. Rhabdomyoma is the most common cardiac tumor diagnosed in fetuses, neonates, and infants and is closely linked to tuberous sclerosis complex [3, 4]. Here we describe an autopsy case of a right ventricular rhabdomyoma in tuberous sclerosis complex.

2.1 *Case report*

The deceased was a 3-month-old male infant. Tuberous sclerosis with a cardiac tumor had been diagnosed before his death. Because the cardiac tumor had not been physically blocking the blood flow, he had not undergone surgical intervention and was under observation. Family members, however, had been told that arrhythmia was possible. The deceased showed no evidence of epilepsy or other neurological manifestation.

On the day before his death, the infant's mother fed him milk and helped him burp at 9:00 PM. He slept covered by a blanket beside his mother. Around 7:30 AM his mother woke up and found him dead, lying on his back. A medicolegal autopsy was

performed on the same day.

2.2 Autopsy findings

The patient's height was 62 cm and his body weight was 6 kg with no significant trauma noted. The autopsy examination revealed no skin lesions related to tuberous sclerosis. The heart weighed 37.3 g with no gross anomalies. It contained 3 mL of dark red blood with fluidity in the left chamber and 12 mL in the right chamber (Fig. 1A). The right ventricle was filled with the tumor (Fig. 1B). No signs of myocardial fibrosis or hypertrophy were found.

The tumor measured 2.1cm ×1.6cm, and was a fusion of multiple tumors with several attachment sites to the myocardium of the free wall and interventricular septum. Small tumor nodules were observed in the interventricular septum. Histologically, vacuolated tumor cells with abundant eosinophilic cytoplasm were observed (Fig. 2A). Abundant glycogen was demonstrated by periodic acid-Schiff staining (Fig. 2B). On immunohistochemical examination, the tumor cells were positive for myoglobin (Fig. 2C) and desmin (supplemental figure) reflecting their myogenous origin and were negative for alpha smooth muscle actin (data not shown). The cells lacked mitotic activity (Ki67 immunohistochemistry, data not shown). To evaluate upregulation of the mammalian target of rapamycin (mTOR) pathway, immunohistochemistry using anti-mTOR antibody was performed and the tumor gave a positive result (Fig. 2D).

The left and right lungs weighed 54.4 g and 67.4 g, respectively. The lungs showed congestion. No heart failure cells or hemosiderin deposit were observed.

Angiomyolipoma was not found in either kidney.

The brain weighed 795.0 g, without hydrocephalus. The cut surface revealed multiple cortical tubers and subependymal nodules along the walls of the lateral ventricle (Fig. 3AB).

2.3 ***Genetic Testing for TSC1 (hamartin) and TSC2 (tuberin) genes***

Genomic DNA was prepared from blood lymphocytes using a genomic DNA extraction kit (Katayama Chemical Industries, Osaka, Japan). All coding regions of the TSC1 and TSC2 genes were screened by CHIPS (CEL nuclease-mediated heteroduplex incision with polyacrylamide gel electrophoresis and silver staining) [5] and base sequences were determined by the direct sequencing method (Fig. 4). In CHIPS analysis of TSC2 Exon10, enzyme mismatch cleavage of PCR products confirmed the presence of cleaved bands. Direct sequencing revealed 1. TSC2 Exon 10 c.1108C>T:p.Gln370* (mutation responsible for the disease), 2.TSC2 Intron 40 IVS40+24G>C (polymorphism in intron), 3.TSC1: Intron 19 IVS19+51A>G (polymorphism in intron without pathological significance), 4.TSC1 Exon 22 c.2829C>T:p.Ala943= (polymorphism without amino acid replacement).

The mutation c.1108C>T:p.Gln370* in the TSC2 gene is a known nonsense mutation reported in non-Japanese patients [6]. Gln370 resides in the TSC1-interacting domain. Referring to the 2012 International Tuberous Sclerosis Complex Diagnostic

Criteria, this case was diagnosed as tuberous sclerosis complex [2]. This case is a sporadic case without family history.

Based on the autopsy results, the immediate cause of death was determined to be a cardiac tumor associated with tuberous sclerosis complex. The tumor was speculated to have grown insidiously. There were no pathological findings in the brain that could directly explain the cause of death.

3. Discussion

Tuberous sclerosis is a genetic multisystem disorder with widespread hamartomas. The affected genes are *TSC1* and *TSC2*, encoding hamartin and tuberin respectively. *TSC1* is located at position 9q34, and encodes a transcript of 8.6 kb, containing 23 exons and encompassing 55 kb of DNA [7]. *TSC2* is located at position 16p13.3, and encodes a transcript of 5.5 kb, containing 41 exons and encompassing 40 kb of DNA [8]. The hamartin–tuberin complex inhibits the mTOR pathway, a downstream signaling pathway involved in various aspects of intracellular functioning including cell growth and proliferation, protein synthesis, and metabolism [1, 9]. A mutation in either *TSC1* or *TSC2* genes interferes with inhibitory control of mTOR, leading to mTOR pathway hyperactivation and cell proliferation [10].

Everolimus is a mTOR inhibitor which has demonstrated efficacy in treating SEGA and renal angiomyolipoma [11]. Everolimus has also proven to be efficacious in size reduction of cardiac rhabdomyomas in cases when surgical resection is not possible

[12]. Comprehensive and reliable screens for *TSC1* and *TSC2* mutations are well-established, and many pathogenic mutations have been identified. In 2012, genetic diagnostic criteria was introduced and identification of a pathogenic mutation in *TSC1* or *TSC2* is now an independent diagnostic criterion and sufficient for a definitive diagnosis [12].

Rhabdomyoma is a benign tumor that occurs in any location in the heart, but is more common in the ventricle. The most common locations are the left ventricle and ventricular septum, although up to 30% are located on the atrial wall or right ventricle [13]. Macroscopically, the tumor is firm, well-defined, non-capsulated, and white or grey, varying in size from millimeters to several centimeters. It can consist of numerous military nodules measuring less than 1 mm. In this instance the term "rhabdomyomatosis" has been used [14, 15]. Microscopically, vacuolated tumor cells with abundant eosinophilic cytoplasm are observed and occasionally a spider cell is noted. Strong reaction with periodic acid-Schiff reagent is observed, reflecting the presence of abundant intracellular glycogen. The tumor usually lacks mitosis, necrosis, or calcification. Immunohistochemical studies document the striated muscle characterization of rhabdomyoma cells, which express myoglobin and desmin [13]. Tumor cells do not express cell proliferation markers such as Ki-67 and PCNA, indicating that the lesions are more likely to be hamartomas than neoplasms [13]. Cardiac rhabdomyoma is closely linked to tuberous sclerosis and observed in 60% of tuberous sclerosis patients [16]. It develops in the fetal stage or early infancy and in

most cases it tends to spontaneously regress [17, 18, 19]. Symptoms resulting from cardiac rhabdomyoma are largely a consequence of tumor size or location within the heart. Surgery is recommended only for patients with refractory dysrhythmias or severe hemodynamic compromise [20]. Surgical resection may be difficult when the tumors are multifocal, infiltrative or giant [21]. Those patients with symptoms have increased risk of sudden death resulting from hemodynamic instability [22].

Cardiac rhabdomyoma is often discussed in relation to sudden, unexpected child death [23-26]. In one study, of 103 primary cardiac tumors causing sudden death, 9 cases (8.7%) were rhabdomyoma [27]. Diffuse cardiac rhabdomyomatosis with or without compensatory hypertrophy was reported in some other sudden death cases [14, 15]. Nonobstructive cardiac rhabdomyoma can also progress to cause right ventricular outflow tract obstruction [28]. In reports from Mayo Clinic (a series of 40 cases), only one death was attributable to obstruction of the ventricular outflow tract by intracavitary tumors [29].

Despite the fact that immunostaining showed no elevated proliferation activity, it was speculated that the tumor had grown insidiously and eventually resulted in obstruction. We speculate that the cause of death was ventricular occlusion by tumor growth.

In this case, brain lesions were also observed. The central nervous system (CNS) is affected in more than 90% of individuals with tuberous sclerosis, characterized by the presence of pathological lesions. These structural CNS lesions are associated with neurological signs and symptoms such as epilepsy and neuropsychiatric disorders [9].

Four common CNS abnormalities are cortical tubers, subependymal nodules, SEGA, and radiologically detectable white matter abnormalities [30]. Cortical tubers are characterized by proliferation of glial and neuronal cells, and loss of the six-layered structure of the cortex. Subependymal nodules are hamartomas, typically seen in the subependymal wall of the lateral ventricles [1]. Brain lesions are not associated with the functional disorder or the cause of death in this case.

This case highlights the need for forensic pathologists to perform a complete autopsy to determine the cause of sudden death when a cardiac tumor is present, including detailed histopathological and genetic examinations.

4. Conflict of interest

The authors declare that they have no conflict of interest.

5. Acknowledgments

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Figure legends

Figure 1 Macroscopic images of the heart.

A: anterior view. B: white tan tumor filling the right ventricular cavity.

Figure 2 Histological images of the cardiac tumor.

A: hematoxylin and eosin staining, B: periodic acid Schiff reaction, C: immunohistochemistry for myoglobin, D: immunohistochemistry for mTOR (mammalian target of rapamycin). Spider cell is noted (AC: Arrow).

Figure 3

Macroscopic and microscopic images of the brain.

A: the cut surface after fixation revealed multiple cortical tubers and subependymal nodules (arrows). B: hematoxylin and eosin staining (subependymal nodules). Large cells similar to gemistocytic astrocytes were noted.

Figure 4

Screening of point mutations by CHIPS and determination of base sequences by direct sequencing method.

Left: post-silver staining CHIPS gel. PCR product of exon 10 of TSC2 gene showed cleaved band (arrow), B: results of direct sequencing of TSC2 exon 10. Nonsense mutation (c.1108C>T:p.Gln370*) was detected.

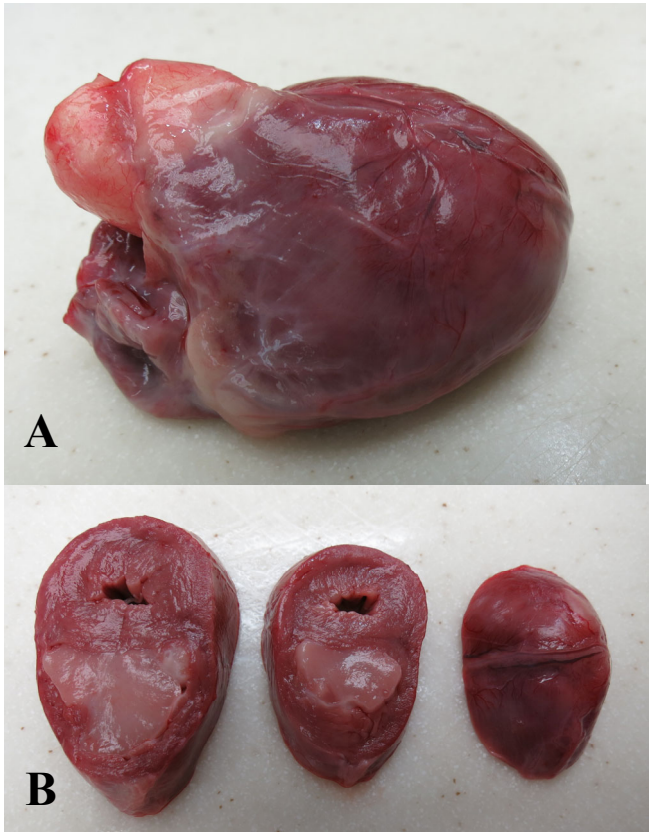


Fig. 1

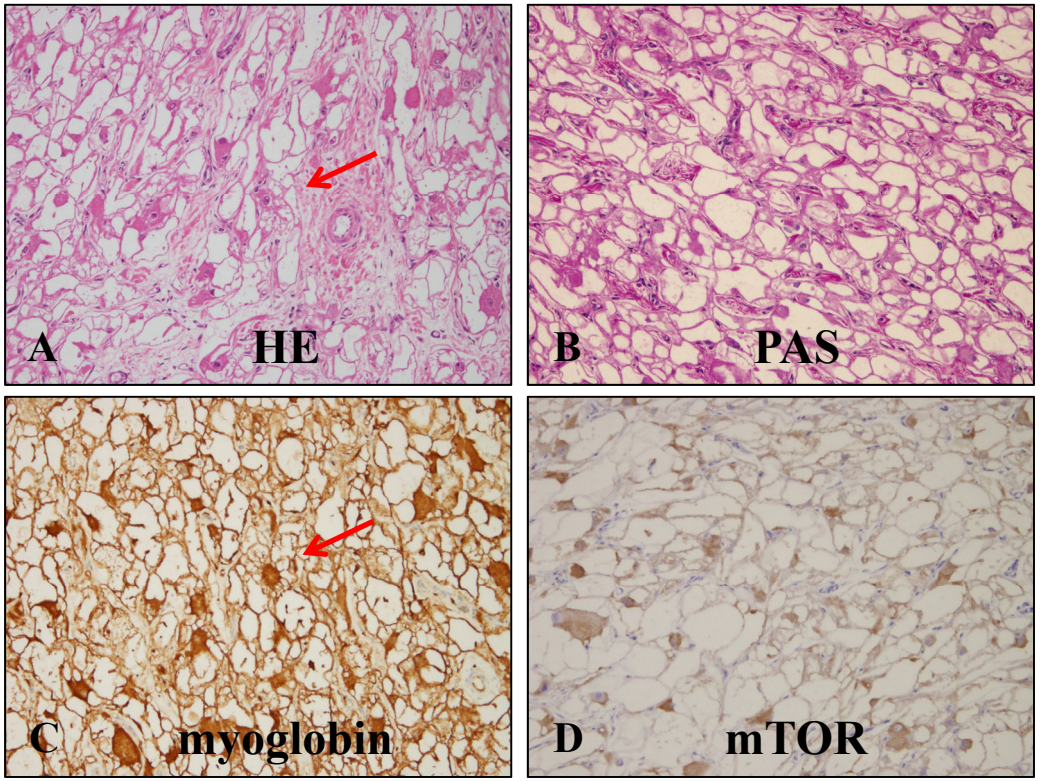


Fig. 2

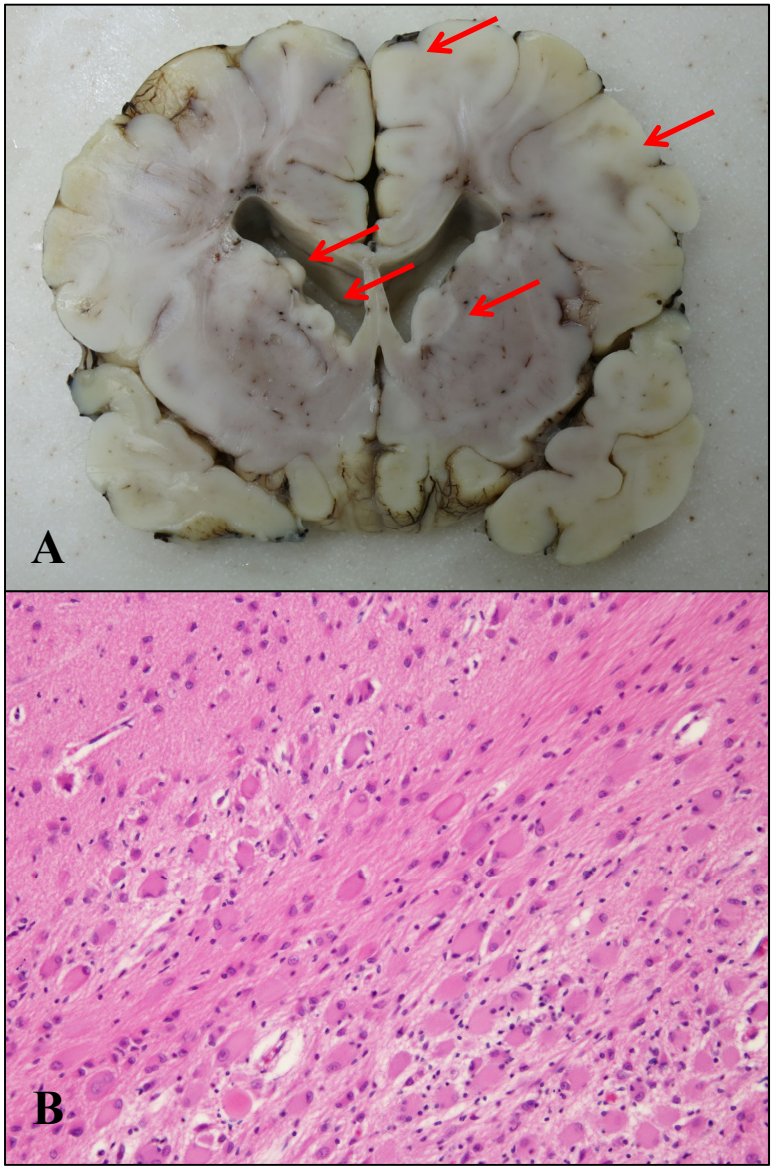


Fig. 3

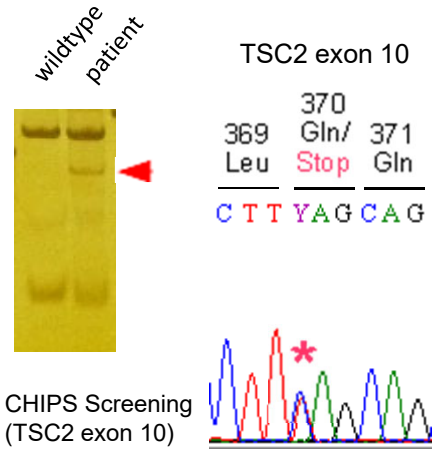
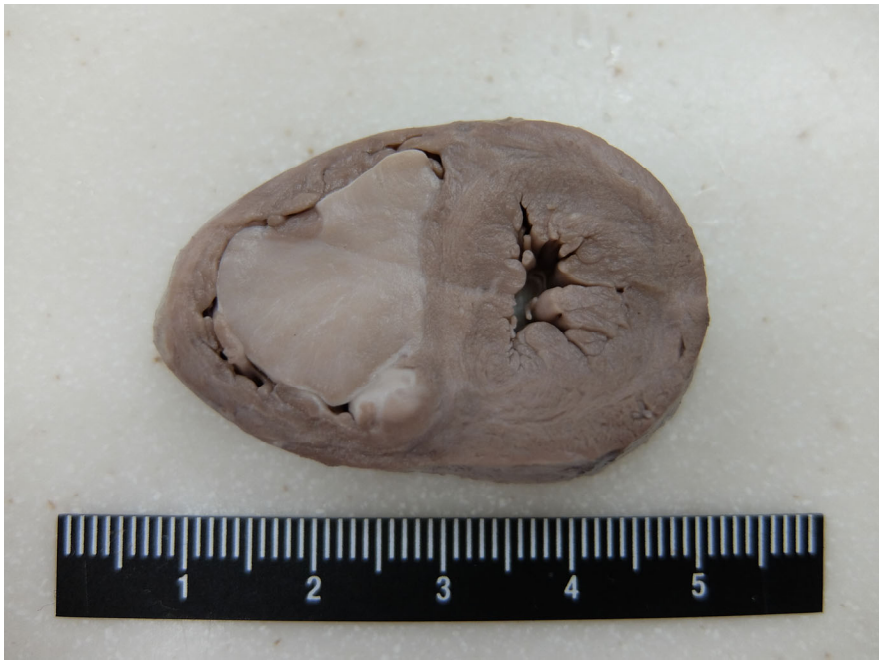
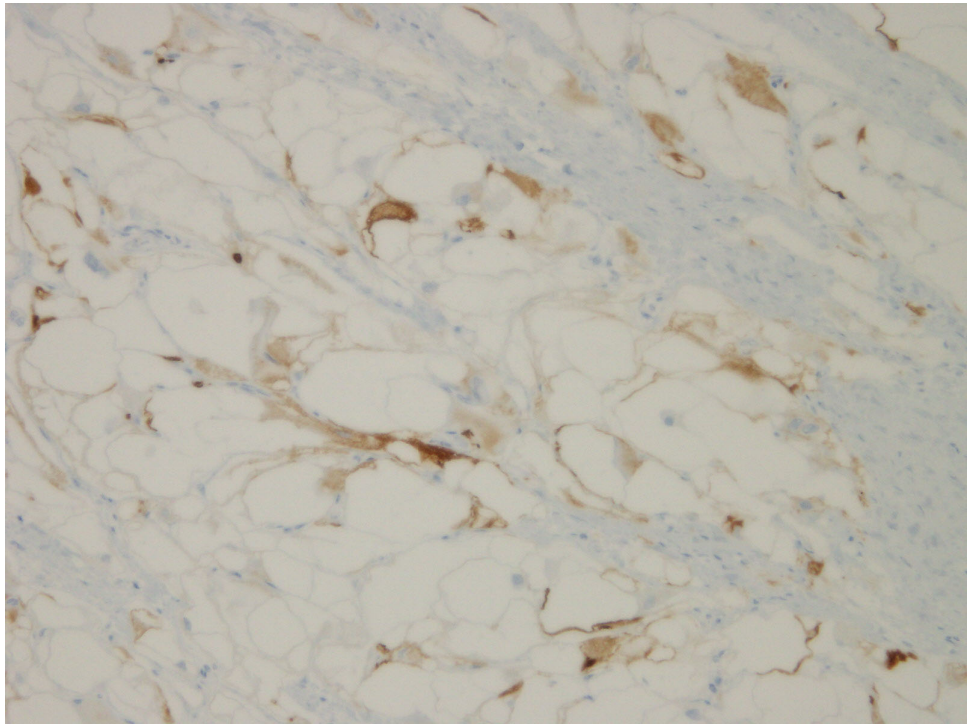

wildtype
patient
TSC2 exon 10
369
Leu
370
Gln/
Stop
371
Gln
C T T Y A G C A G
CHIPS Screening
(TSC2 exon 10)

Fig. 4



Supplemental Figure 1
Macroscopic finding of the myocardium (after fixation).



Supplemental Figure 2
Immunohistochemistry of cardiac rhabdomyoma.
The tumor cells were positive for desmin.