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Antenatal screening and diagnosis of tuberous sclerosis complex by fetal echocardiography and targeted genomic sequencing

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Abstract

Although fetal cardiac rhabdomyoma can be the initial finding in patients with tuberous sclerosis complex (TSC), the challenges of precise genetic diagnosis of TSC can now be potentially overcome by new whole or targeted genomic sequencing. The goals of this study were to investigate the correlation between suspected cardiac rhabdomyoma and TSC to provide the information for a prenatal diagnosis of TSC and to validate the TSC genotype in this cohort of fetuses with suspected prenatal cardiac rhabdomyoma.

We retrospectively analyzed 10,728 fetal echocardiograms from January 2013 to March 2016 in our institution. A total of 32 fetuses were suspected of having cardiac rhabdomyomas. Among them, 15 subjects met the inclusion criteria and provided written consent. Samples from fetuses (n = 13 after terminations) and newborns (n = 2) were available for targeted genomic sequencing of the exons and introns of the TSC1 and TSC2 genes and the adjacent 10 base pairs and for validated studies using Sanger sequencing.

Among the 15 subjects with suspected cardiac rhabdomyoma and TSC genomic sequencing data, 7 subjects were familial and 8 subjects were sporadic cases. Fetal echocardiography showed a single tumor in 2 fetuses and multiple tumors in 13 fetuses. Gene sequencing analysis showed no mutation of the TSC1 or TSC2 gene in 2 subjects with a single tumor but positive mutations in all 13 subjects with multiple tumors. Among the latter, 5 mutations were "pathogenic" and have been previously reported (4 familial and 1 sporadic). Six new mutations were "likely pathogenic" and had not been previously reported (3 familial and 3 sporadic); 1 was of "uncertain significance" (sporadic), and 1 was suspected of being "likely benign" (sporadic).

Prenatal suspected cardiac rhabdomyoma detected by fetal echocardiography should raise the suspicion of TSC. In fetuses with multiple tumors, especially the familial cases, genomic sequencing has a high yield of detecting TSC-causing genes. Patient history, prenatal fetal echocardiography, and targeted genomic sequencing may facilitate screening for, diagnosis of, and counseling for TSC.

Abbreviations: bp = base pair, MRI = magnetic resonance imaging, TSC = tuberous sclerosis complex.

Keywords: cardiac rhabdomyoma, fetal echocardiography, TSC genomic sequence, tuberous sclerosis

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder affecting 1/6000 to 10,000 live births. It is characterized by the growth of benign hamartomas in multiple-organ systems like the brain, the kidneys, the heart, and the skin.[1,2] Clinical manifestations include seizures, behavioral problems, learning difficulties, cardiac tumors, and skin lesions. Precise prenatal screening for and diagnosis of TSC are important for prognosis, a decision on pregnancy outcome, and counseling.

The first manifestation of TSC is often the identification of a cardiac tumor(s) by fetal ultrasound.[3,4] Cardiac tumor(s) or rhabdomyoma (s) can occur in more than 50% of patients with TSC. Subsequently, clinical confirmation of the diagnosis of TSC by phenotypic findings alone is challenging in patients with cardiac tumors.

Recently, it has been shown that mutations in at least 2 genes (i.e., TSC1 and TSC2) can cause TSC, making precise prenatal diagnosis of TSC possible. Therefore, the specific aims of this study were to investigate the correlation between suspected cardiac rhabdomyoma and TSC to provide the information for the prenatal diagnosis of TSC and to validate the TSC genotype in this cohort of fetuses with suspected prenatal cardiac rhabdomyoma.

1. Materials and methods

1.1. Clinical data

A total of 10,728 fetuses were evaluated from January 2013 to March 2016 at the Beijing Anzhen Hospital, a regional and national referral center for cardiovascular diseases.

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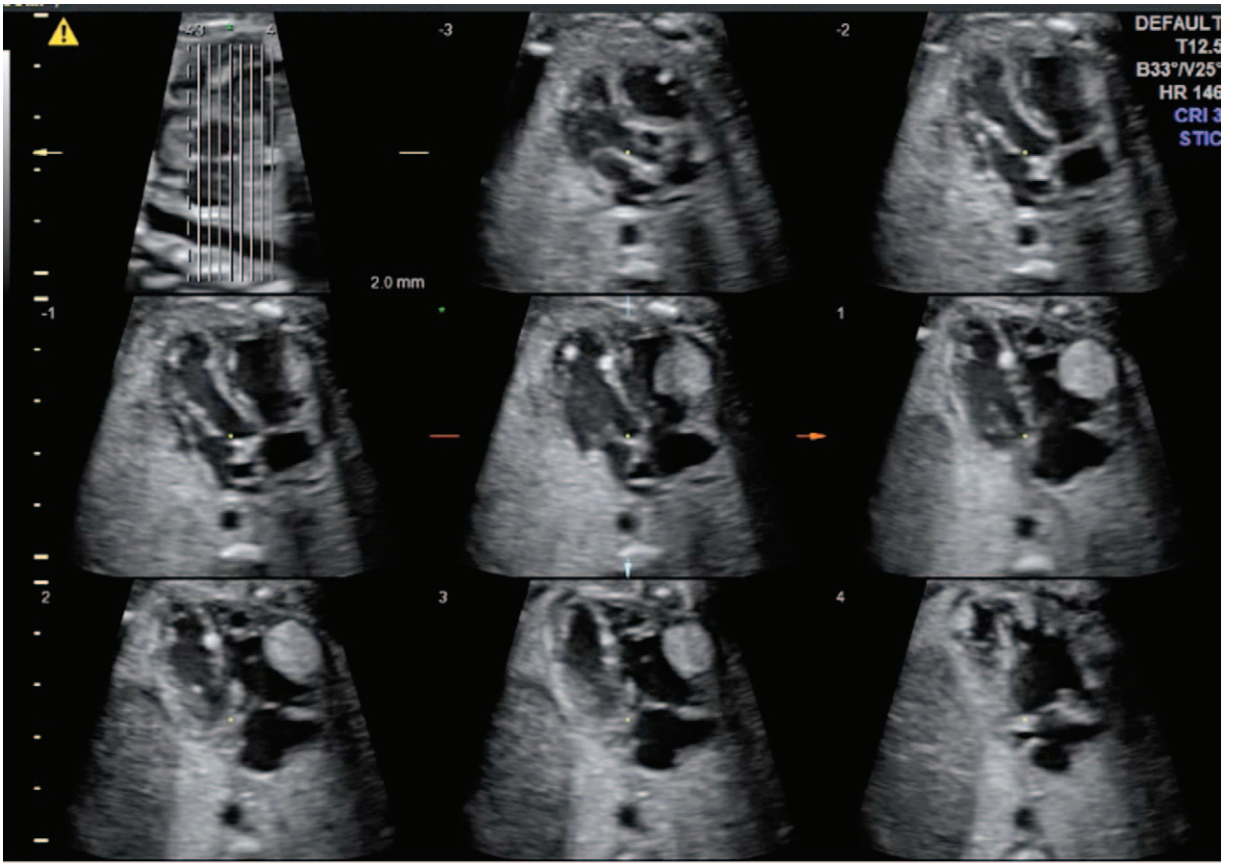

Figure 1. Fetal echocardiography (spatiotemporal image correlation, tomographic ultrasound imaging mode) showing multiple cardiac tumors (patient no. 3).

The study inclusion criteria were suspected cardiac rhabdomyomas detected by fetal echocardiography; homogeneous hyperechogenic oval tumor(s) located in the cardiac chamber, the interventricular septum, the ventricular wall, or the atrial wall (Fig. 1); and the absence of color Doppler flow within the tumor body.[3] Informed written consent was obtained for tissue or blood samples for each genetic study.

Thirty-two fetuses had suspected cardiac rhabdomyomas. Among them, 15 fetuses met the inclusion criteria with all clinical, fetal echocardiographic, and TSC genomic sequencing data. Thirteen fetuses were terminated, and 2 fetuses were delivered. The mean maternal age was 26.4 ± 5.3 years (range 19–38 years), and the mean gestational age was 29.4 ± 4.7 weeks (range 24–37 weeks).

The ethics committee of Beijing Anzhen Hospital approved the study. Informed consent was obtained from all expecting parents of the study subjects. Samples of fetal umbilical cord, deltoid muscle tissue, and parental peripheral blood were obtained for each of the terminated pregnancies (n = 13). For the delivered fetuses (n = 2), peripheral blood samples were also collected from the newborns (n = 2) and their parents.

1.2. Equipment

Complete fetal echocardiograms, including 2-dimensional, color, and pulse Doppler, were performed according to the guidelines of the American Society of Echocardiography.[5] All ultrasound examinations were performed by experienced operators utilizing real-time scanning using a 3.5-MHz curved-array transducer and a 2 to 5 Hz curvilinear transabdominal 3-dimensional transducer. Spatiotemporal image correlation and tomographic ultrasound imaging were also obtained using a Voluson E8 system (GE Healthcare Ultrasound, Milwaukee, WI).

1.3. Targeted genomic sequencing

Genomic DNA was extracted from tissue and blood samples using the TIANamp DNA Kit (DP318) (Tiangen, China). Genomic DNAs that were not degraded, a total of 2 μg, with a concentration of at least 50 ng/μL, were eligible. A 1 μg-solution of genomic DNA was sheared into small fragments, ranging from 200 to 300 base pairs (bp), by sonication. After end-repairing, "A"-overhanging, and adapter-ligation, the fragments underwent 10 cycles of polymerase chain reaction with index primers. Following the purification, the library was sequenced on the Illumina Hiseq2500 system (PE 50 bp reads). Approximately 15 Mb of unique reads were produced for each sample. Targeted Next-Generation Sequencing detects TSC1 and TSC2 gene exons, introns, and adjacent 10 bp.

Sanger sequencing was carried out both in fetuses and in parents. Analyses of the genetic results were based on the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://decipher.sanger.ac.uk/), and OMIM (http://www.ncbi.nlm.nih.gov/omim). TSC1/TSC2 genetic variants were classified into 5 categories: "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign."[6]

2. Results

Clinical, fetal echocardiographic, and targeted genomic sequencing data are shown in Table 1. All except 3 patients were gravid 1, para 0. Patients nos. 3 and 6 were gravid 2, para 0; both had a

Table 1

General clinical, fetal echocardiographic, and targeted genomic sequencing findings.

No.	Age	GW	Family history	CR character	Complications	Genetic testing	Sanger sequencing	Outcome
1	37	37	–	RV, isolated	—	—		Birth
2	23	24	–	LV, isolated	PE, LV compression	—		Termination
3	28	30	+	LV + RV, multiple	—	TSC2: c.4813C>T (nonsense mutation)	Pregnant women: TSC2: c.4813C>T	Termination
4	22	28	+	LV + RV, multiple	VSD	TSC2: c.682C>T (nonsense mutation)	Pregnant women: TSC2: c.682C>T	Birth
5	23	26	+	LV + RV, multiple	TR	TSC2: c.5228G>T (missense mutation)	Pregnant women: TSC2: c.5228G>T	Termination
6	28	35	+	LV + RV, multiple	TR	TSC2: c.2125G>T (frameshift mutation)	Pregnant women and her mother: TSC2: c.2125G>T	Termination
7	24	24	+	LV + RV, multiple	MV obstruction	TSC2: c.1939G>A (missense mutation)	Pregnant women: TSC2: c.1939G>A	Termination
8	19	24	+	LV + RV, multiple	LVOT obstruction	TSC2 c.2251C>T (nonsense mutation)	Pregnant women: TSC2 c.2251C>T	Termination
9	25	24	+	LV, multiple	—	TSC2: c.1139T>C (missense mutation)	The father TSC2: c.1139T>C	Termination
10	30	34	–	LV + RV, multiple	—	TSC1: c.1525C>T (nonsense mutation)	The parents (–)	Termination
11	38	32	–	LV + RV + LA, multiple	—	TSC2 c.3599G>C (missense mutation)	The parents (–)	Termination
12	23	31	–	LV + RV, multiple	Hydrops	TSC2: c.557delT (frameshift mutation)	The parents (–)	Termination
13	23	36	–	LV + RV, multiple	—	TSC2: c.4728delG (frameshift mutation)	No peripheral blood of the parents available	Termination
14	26	26	–	RV + RA, multiple	TR	TSC2: c.2447C>T (missense mutation)	No peripheral blood of the parents available	Termination
15	27	30	–	LV + RV, multiple	LVOT + RVOT obstruction	TSC2: c.2717_2725dupAGGATTTTG (frameshift mutation)	The parents (–)	Termination

CR = cardiac rhabdomyomas, GW = gestational week, LV = left ventricle, LVOT = left ventricular outflow tract, MV = mitral valve, PE = pericardial effusion, RA = right atrium, RV = right ventricle, RVOT = right ventricular outflow tract, TR = tricuspid regurgitation, TSC = tuberous sclerosis complex, VSD = ventricular septum defect.

history of termination of their first pregnancy due to multiple cardiac rhabdomyomas in the fetus. The third patient (no. 10), who was gravid 3, para 1, had a healthy, 6-year-old daughter; the results of TSC Sanger sequencing were negative. She opted for termination of the second pregnancy because the fetus had stopped growing with likely fetal demise. Fetal echocardiography taken during the third pregnancy showed that the fetus had multiple cardiac tumors. In this cohort, 2 fetuses had single isolated tumors and 13 had multiple tumors (Fig. 1). Fetal complications included 1 with both left ventricular and right ventricular outflow tract obstruction, 1 with left ventricular outflow tract obstruction, 1 with mitral valve obstruction, 3 with mild tricuspid regurgitation, 1 with mild pericardial effusion, and 1 with fetal hydrops (pericardial effusion, pleural effusion, and ascites).

Tissue or blood samples from 15 fetuses or newborns underwent targeted genomic sequencing to detect TSC1 and TSC2 mutations. Gene sequencing analysis showed no mutation of TSC1 or TSC2 in 2 subjects with a single tumor but positive mutations in 13 subjects with multiple tumors. The data are summarized in Fig. 2. All the terminated fetuses (n = 13) were autopsied; the autopsy results confirmed the diagnosis of cardiac rhabdomyoma.

In 13 subjects with multiple tumors, 7 were familial and 6 were sporadic cases. Five mutations were "pathogenic" and had been previously reported (4 familial and 1 sporadic); 6 new mutations were "likely pathogenic" and have not been previously reported (3 familial and 3 sporadic); 1 was of "uncertain significance" (sporadic); and 1 was "likely benign" (sporadic).

In the familial cases, the husbands of 6 pregnant women and 1 pregnant woman had medical and/or familial histories of TSC. Images of autopsied and pathological specimens from Family 1 (patient no. 3) are shown in Figs. 3 and 4. The fetus had c.4813C>T nonsense mutations in TSC2, which was also

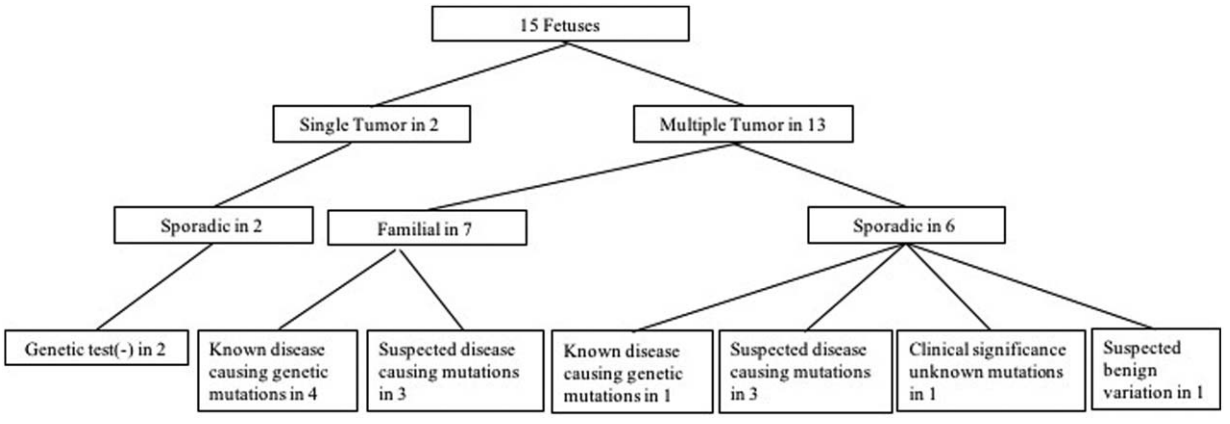

Figure 2. The flow chart of this cohort.

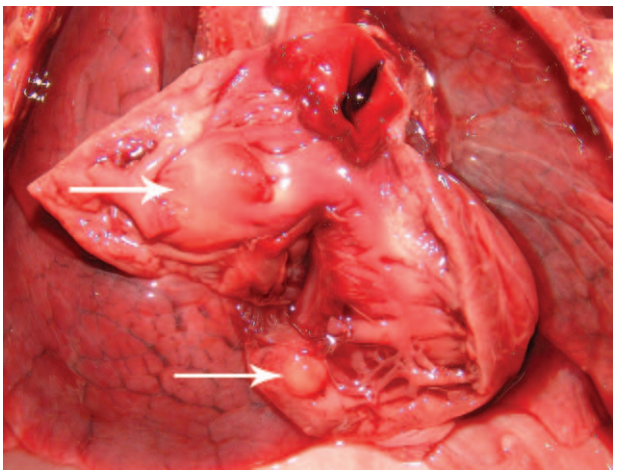

Figure 3. Specimens of fetal cardiac rhabdomyoma (same patient as in Fig. 1). The right ventricle is open and multiple cardiac tumors (arrows) are present.

confirmed in the mother using Sanger sequencing. In Family 2 (patient no. 4), the fetus had c.682C>T nonsense mutations in TSC2, also confirmed in the mother using Sanger sequencing. In Family 3 (patient no. 5), the fetus had c.5228G>T missense mutations in TSC2, also confirmed in the mother using Sanger sequencing. In Family 4 (patient no. 6), the pregnant woman was diagnosed as having TSC. Her first pregnancy was terminated for multiple fetal cardiac tumors, without genetic testing. Her deceased maternal aunt had a history of epilepsy. The fetus had c.2125G>T missense mutations in TSC2, also confirmed in the mother using Sanger sequencing. Also, this mutation was detected in the maternal mother. In Family 5 (patient no. 7), the fetus had TSC2: c.1939G>A missense mutations, also confirmed in the mother using Sanger sequencing. In Family 6 (patient no. 8), the fetus had TSC2 c.2251C>T nonsense mutations that were also confirmed in the mother using Sanger sequencing. In Family 7 (patient no. 9), the fetus had c.1139T>C missense mutations in TSC, also confirmed in the father of the fetus using Sanger sequencing.

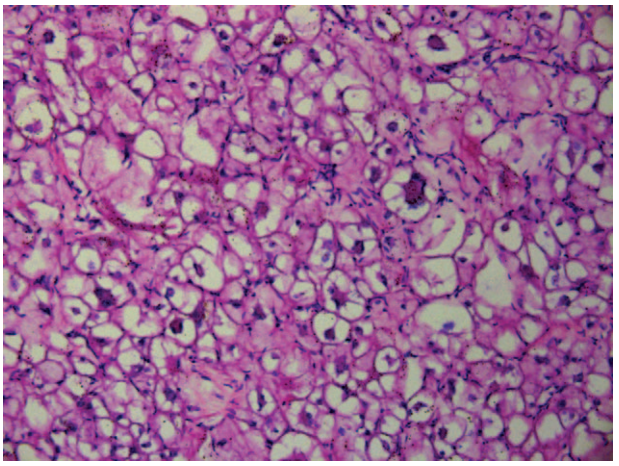

Figure 4. The pathological study of the cardiac rhabdomyoma (same patient as in Figs. 1 and 2): hematoxylin and eosin stain (20 × 10), showing vacuolar, translucent, and eosinophilic cytoplasm in cells or the "spider cell."

In the 6 sporadic cases, TSC genetic testing results from the parents were all negative, and the fetuses had de novo mutations. In patients nos. 10, 11, 12, 13, 14, and 5, the mutations were TSC1: c.1525C>T nonsense mutations, TSC2: c.3599G>C missense mutations, TSC2: c.557delT frameshift mutation, TSC2: c.4728delG frameshift mutation, TSC2: c.2447C>T missense mutation, and TSC2: c.2717_2725dupAGGATTTTG frameshift mutation, respectively.

The genetic mutations can be divided into 4 categories: Pathogenic genetic mutations were detected in 4 familial cases and in 1 sporadic case (patients nos. 3, 4, 7, 8, and 10). "Likely pathogenic" mutations were found in 6 cases: 3 were familial and 3 were sporadic cases (patients nos. 5, 6, 9, 11, 12, and 13). "Uncertain significance" mutations were found in 1 sporadic case (patient no. 14). "Likely benign" mutations were identified in 1 sporadic case (patient no. 15).

Two fetuses were delivered. One of them had a single tumor (patient no. 1), and the other had multiple tumors (patient no. 4). Postnatal follow-up echocardiography was performed when they were 2 years of age and 1 ½ years of age, respectively. No tumor regression was observed, but neither baby had any signs or symptoms or required any intervention.

3. Discussion

Our study demonstrated that it is feasible to provide prenatal screening, a diagnosis of TSC, prognosis, and counseling of expectant families by an integrated approach of clinical, fetal ultrasound, and targeted genomic sequencing.

Phenotypically, TSC is characterized by the growth of benign hamartomas in multiple organ systems such as the brain, the kidneys, the heart, and the skin. Cardiac rhabdomyomas are often the first clinical manifestation of TSC in the fetal period. Ultrasound screening in the second trimester allows us to identify cardiac rhabdomyomas, but it remains challenging to detect these benign hamartomas in other organs such as the brain, the kidneys, the heart, and the skin. Fetal magnetic resonance imaging (MRI) is useful in detecting extracardiac hamartomas but is difficult to use as a screening tool.

With the advent of genomic sequencing, at least 2 genes, TSC1 and TSC2, have been identified as a cause for TSC. The disease is caused by mutations in either the TSC1 gene on chromosome 9q34[7] or the TSC2 gene on chromosome 16p13.3.[8]

Most TSC genetic testing was done during infancy, childhood, or adulthood. Very few screenings included fetal echocardiography and TSC genetic sequencing. A family history of TSC is an important clue in the diagnosis of fetal TSC. In this study, 7 of the 15 cases had a family history of TSC and were confirmed with likely TSC1 or TSC2 mutations by targeted sequencing of the fetal or neonatal genomes. Suspected diagnosis of cardiac rhabdomyomas by fetal ultrasound screening and fetal echocardiography is often crucial to the prenatal diagnosis of TSC. Fetal cardiac rhabdomyomas can be located in any part of the heart.[9] Many fetal echocardiographic studies support the notion that multiple tumors in the ventricular myocardium are often rhabdomyomas.[10,11] The rhabdomyomas may be located in the interventricular septum, left and right atria, and the ventricular myocardium. The ultrasound characteristics of these tumors are hyperechogenicity, clear demarcation, and the presence of ovoid hamartomas. Cardiac rhabdomyomas usually occur late during the gestational period, and the size and the number may increase during gestation. Cardiac rhabdomyomas

were first detected as early as 24 weeks of gestation in this study. Serial follow-up in the 3rd trimester, even if the results of the early studies were negative, is warranted in high-risk subjects, such as those with a family history of TSC. It has to be noted that the fetus will still have a high risk of TSC even if no cardiac rhabdomyomas are detected. Fetal complications of cardiac rhabdomyosarcoma include arrhythmias, outflow obstruction, pericardial effusion, cardiac compression, and fetal hydrops.[12] Although cardiac rhabdomyomas tend to regress after birth in most cases, cardiac surgery is indicated when severe complications such as hemodynamically significant obstruction or uncontrollable ventricular arrhythmia occur.

Unfortunately, the fetal echocardiographic characteristics alone are not sufficient to ascertain the presence of mutations that cause TSC. In this cohort, although TSC1 and TSC2 were negative in both cases with a single isolated cardiac rhabdomyoma, multiple cardiac rhabdomyomas were either "pathogenic" or "likely pathogenic" TSC1 and TSC2 mutations (n = 11) or mutation or variation with "uncertain significance," or "likely benign" (n = 2). Targeted sequencing may assist in the ascertainment of TSC in these cases.

In our studies, 5 TSC mutations were detected as "pathogenic" and previously described in other patients,[13–16] and 4 were familial cases. The Sanger sequencing analysis showed that, in these 4 familial cases, the mothers and fetuses had the same genetic mutations in the TSC2 gene. Four mothers had symptoms suggestive of TSC. In the sporadic case, the mutation was in TSC1. De novo "likely pathogenic" mutations were identified in 6 cases: 3 of them were familial and 3 were sporadic cases. In the 3 familial cases, the Sanger sequencing analysis showed that 2 mothers and their fetuses had the same genetic mutations in the TSC2 genes; 1 father and 1 fetus had the same genetic mutations in the TSC2 genes, in which mutations had not been previously reported in TSC patients. Both mothers had symptoms suggestive of TSC, but the fathers denied the symptoms. Although no cases have been reported, the mutations in the 3 sporadic cases may lead to early termination of protein synthesis, and the mutations were located before the previously reported disease-causing mutation.[15] Thus, we speculated that the mutations were de novo "likely pathogenic" mutations. Finally, there was 1 variant with "uncertain significance" and 1 variant with "likely benign" variation; both were sporadic cases. The case with "likely benign" variation was previously reported in patients with sporadic TSC.[17,18] It has also been reported that, in a familial case, the father carried the same variant but had no phenotypical manifestations of TSC.[19] Thus, we speculated that the variation was benign.

In summary, our study not only confirmed 5 known "pathogenic" TSC-causing gene mutations but also detected 6 "likely pathogenic" mutations. The integrated clinical, fetal echocardiographic, and targeted genomic sequencing approach should facilitate prenatal screening and the diagnosis of TSC.

This study has a few limitations. TSC is a rare disease. Although we had a large fetal cohort, the sample size for TSC with genomic sequencing data is small. We did not perform fetal MRI studies, which may help in the prenatal screening and diagnosis of TSC. However, fetal MRI is limited to a few large academic centers. Furthermore, fetal ultrasound and fetal echocardiography are the fetal imaging modalities of choice and are widely and routinely performed. An integrated clinical, fetal echocardiographic, and targeted genomic sequencing approach is likely to be more feasible and effective than a fetal MRI-based approach. Finally, we have not validated the mutations and variations that were not ascertained by previous reports. Future functional translational animal studies and/or clinical trials are necessary to confirm causality of these mutations and variations for TSC.

In conclusion, our study validated an integrated clinical, fetal echocardiographic, and targeted genomic sequencing approach for more precise prenatal screening and the diagnosis of TSC. Family history and multiple cardiac tumors are 2 high-risk factors for TSC and may necessitate targeted genomic sequencing of TSC1 and TSC2 genes. This approach may improve the efficacy of prenatal detection of TSC and facilitate prognosis, counseling, and potential early intervention to improve the outcomes of these individuals.

Author contributions

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Writing – review & editing: Shuping Ge.

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