A Multidisciplinary Approach in Prenatal Diagnosis of TSC With Cardiac Rhabdomyoma as the Initial Symptom

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Primary research

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Abstract

The long-term prognosis of fetus with cardiac rhabdomyoma (CR) depends on the correlation with tuberous sclerosis complex (TSC). Recent years, numerous variations of uncertain significance (VUS) of TSC genes produced by high-throughput sequencing makes counseling challenging, studies until now tend to sidestep the tricky topics. Here, we integrated detailed parental phenotype, echocardiography, neuro MRI and genetic information to conduct a comprehensive evaluation for 61 CR fetuses. As a result, multiple CRs and cerebral lesions appeared in 90% and 80% respectively in fetuses with pathogenic (P)/likely pathogenic (LP) TSC1/TSC2 variation. 85.7% of the live-born with P/LP presented TSC-associated signs. 85.7% VUS without nervous findings had good prognosis. Genetic evidence and cerebral MRI findings are the most sensitive index to assess long-term prognosis, which complement and confirm each other for a TSC diagnosis. 68.9% fetuses with CR could benefit from this multidisciplinary approach, which turned to be potentially clinically actionable with precise clinical/genetic diagnosis or had a foreseeable outcome. Our practice provides a practical and feasible solution for perinatal management and prognostic guidance for fetuses with CR.

Introduction

Cardiac rhabdomyoma (CR) is one of the most common primary cardiac tumors in the fetus, with an estimated incidence of 1/6,000 live births [1]. An unprecedented increase in CR benefited from the rapid development of fetal echocardiography in recent decades. However, it is still challenging to accurate prognostic information of these newborns.

As a benign hamartoma, the prognosis of CR without severe complications is typically good [2]. But CR has uncertainty and occurs anywhere in the heart, which may result in arrhythmias, effusions, fetal hydrops, and even stillbirth. Noteworthy, to some extent, CR is a characteristic sign and an initial symptom for tuberous sclerosis complex (TSC) prenatally, which has a high possibility of neurological development disorder. Reported rates of TSC in fetal with CR vary from 50%-90% [3–4]. Approximately 90% of infants with TSC experience infantile spasms, seizures, mental retardation, or autism [5]. As a multisystem genetically based neurocutaneous syndrome, TSC inherited in an autosomal dominant manner. Cortical tubers and subependymal nodules (SEN) are typical cerebral lesions with a high risk of obstructive hydrocephalus, which are the leading cause of mortality. There is a sparse report about the intrauterine brain damages of TSC, and the relationship with imaging phenotype and genotype is not clear yet.

TSC1/TSC2 mutations are the vital disease-causing genes and recognized as independent and sufficient diagnostic criteria, even in the condition of no visible typical clinical signs [6]. With the application of NGS technology, the number of identified variants increasing explosively, meanwhile massive VUS coming behind. 3446 TSC1 and 8112 TSC2 variations have been recorded in the Tuberous Sclerosis Database (http://chromium.lovd.nl/LOVD2/TSC/home), including 268 VUS of TSC1 and 642 VUS of TSC2. It was showing a broad spectrum of variations without conspicuous hotspots in patients. Mutation types
almost equal coverage insertions/deletions/duplications, frameshift, nonsense, missense, splicing, sometimes encounter deep intron $^7$. It is implying that no typical mutation type was well tolerated. Disproportionately, limited understanding of the role and lack of awareness of genotype-phenotype correlations excessively disturbs our judgment of the pathogenesis with these VUS, which is hugely critical for fetal prognosis, informed reproductive choices and evidence-based perinatal management.

The unpredictability of the fetal CR and the uncertainty of variations with $TSC1/TSC2$ genes make phenotype and prognosis prediction of TSC clinically challenging. Here, we revealed complete imaging and the molecular portrait of a fetus with CR(s) to date, adding multi-information for a comprehensive evaluation and proposing the clinical workflow for clinical management.

**Materials And Method**

**Patient recruitment and sample collection**

The study was approved by the institutional review board of the ethics committee in Guangdong Women and Children Hospital[201601046]. Pregnant women who suspected fetal CR by the second-trimester screening were recruited in our center from March 2014 to November 2019. All fetuses underwent echocardiography to evaluate the properties of tumors, and part of them carried out MRI scans of cerebral to explore other clinical evidence. To confirm the clinical phenotype, a comprehensive clinical examination was also concurrently performed in parents and/or relevant patients. Diagnostic criteria we referred to is the international recommendations of TSC revised in 2013 $^6$.

Fetal samples collection depended on the gestational age at the time of the invasive prenatal exam, with amniocentesis (16–24 weeks) or cordocentesis (more than 24 weeks), respectively. Karyotype and SNP-array were performed simultaneously with NGS or before. All the fetuses accompanied with chromosome aneuploidy or copy number variations (CNVs) were excluded. For each relevant family member, five-milliliter peripheral blood samples were draw and stored in the ethylenediaminetetraacetic acid (EDTA) anticoagulation vacuum tube. Skin or cardiac tissues obtained through aborted fetuses were all signed informed consents with the enrolled families.

**Fetal Echocardiography and other imaging examinations**

According to the "ISUOG Practice Guide: Fetal Heart Ultrasound Screening (Updated Version)," comprehensive use of two-dimensional ultrasound and echocardiography by GE Voluson E8 color Doppler ultrasound system (GE Medical Systems, Zipf, Austria) which equipped with a 2–5 MHz two-dimensional convex array transducer and a 4-8.5 MHz three-dimensional transducer. Record the tumor number, location, size, echo, relationship with surrounding tissues, heart rhythm, and hemodynamic changes in details, as well as fetal brain, spine, chest, abdomen, limbs and other structures.

**Following-up**
Continuing pregnancies were observed, and newborns with CRs were born at the author's center or the referral cooperation. All born children are followed up by a pediatric expert team which including a pediatric cardiologist, a pediatric neurologist, an imaging expert, and a genetic counselor. Termination of pregnancy (TOP) or obtaining a diagnosis (clinically or/and genetically) as the endpoint of clinical observation. Postnatal examinations included clinical examination, echocardiography, and MRI scanning if suspicious extracardiac complications are found.

Next-Generation Sequencing

Genomic DNA samples were extracted using the Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Sequencing of \( TSC1 \) gene and \( TSC2 \) gene in Trios was performed with a custom-designed Roche Nimblegen (Madison, WI) (SeqCap EZ Choice Library) chip capturing the \( TSC1 \) (NM_000368, exon 3–23, exons 1 and 2 are UTRs) and \( TSC2 \) (NM_000548, exon 2–42, exons 1 UTRs) genes followed by manufacturer's protocols (JIAJIAN-Clinical Laboratories, BEIJING, China). The found variants were further verified by Sanger sequencing in fetuses and parents. Analysis of genetic results is based on the genomic variation database (http://dgv.tcag.ca/dgv/app/home), DECIPHER database (https://decipher.sanger.ac.uk/), and OMIM database (http://www.ncbi.nlm.nih.gov/omim). Variant-classification based on the guidelines of the ACMG and divided into five categories: "I: Pathogenic [P]," "II: likely pathogenic [LP]," "III: variants of uncertain significance [VUS]," "IV: Likely Benign [LB]" and "V: benign [B]."

Results

General information and imaging results prenatally

A total of 61 fetuses with CR were included in the study. The median age of pregnant women was 28 (range 22 ~ 38) years. The average GA of CRs detected during routine scans was \( (25 \pm 3.5) \) weeks (range 19 ~ 34). There were 59 singleton pregnancy and two dichorionic twin pregnancy, in which one of the fetuses presented CR. Sonographic images showed round, hyperechogenic, homogenous masses with clear boundaries and absence of color Doppler flow inner. Solitary and multiple rhabdomyomas were detected in 18 fetuses (29.5%) and 43 fetuses (70.5%), respectively (Fig. 1a,1b). All CRs were distributed in the ventricle, interventricular septum, and the atrium, with the most common left ventricle and ventricular septum. Tumor size was recorded in 59 cases, and, of these, the median diameter was 8.1 mm (range 2.2 ~ 31.8). Forty-nine fetuses have more than one echocardiography monitoring records. Throughout pregnancy, 41% of the 49 fetuses had tumors increased, 7 cases kept stable, and only one had somehow subsided. Echocardiographic findings were summarized in Table 1.

Severe hemodynamic changes occurred in 6.6% (4/61) of the fetuses. Six fetuses presented left/right ventricular outflow tract obstruction, and three of them progressed to pericardial and peritoneal effusion (Fig. 1c). Moderate tricuspid regurgitation with or without pulmonary hypertension was observed in two fetuses (Fig. 1d). In cases 6, the gravida had been diagnosed as TSC clinically, she underwent sonographic screening ahead of schedule at 16 weeks of GA. The fetal appeared ventricular hyper echo
accompanied by severe congenital cardiac structural deformities, which directly related to a poor perinatal outcome rather than a TSC-associated prognosis. All subjects did not detect arrhythmia throughout pregnancy. MRI was performed from 20 to 36 weeks of gestation in 53 fetuses, and 25 fetuses appeared SENs and cortical tubers. Three cases showed lateral or bilateral ventriculomegaly. Detailed information on these cases was displayed in Table 2.

Detection of TSC1 and TSC2 variants

In general, 45 of 61 fetuses (73.8%) were detected TSC1 and TSC2 variants, leaving 16 no variant identified (NVI). Two-thirds of these variants were de novo. Roughly 50% of these variants had not been reported previously. Following ACMG Standards, we identified 30 variants of P/LP and 16 VUS (Fig. 2d). The P/LP variant spectrum in TSC1/TSC2 was 16 (34%) nonsense, 11 (23.4%) missense, 7 (14.9%) frameshift, 5 (10.6%) splice, 4 (8.5%) indels, and 2 (4.3%) introns (Fig. 2e). When focused on VUS, poor prognosis presented in all the truncating variants fetus. Half of the missense/indel variants and all the intron variants tended to have a good outcome (Fig. 2f).

Genotype-phenotype correlation

The profile of genotype and phenotype in fetal with CR(s) was depicted in Table 3. For the 30 cases identified with variations of P/LP: 90% (27/30) had multiple CRs. 80% (20/25) showed cerebral MRI positive before birth, and TSC-associated signs appeared in 85.7% (6/7) of the live-born. Two babies with multiple CRs but normal brain MRI were born, one of which developed epilepsy at six months, and SENs appeared. In the fetuses carrying VUS: the incidence rate of multiple tumors was as high as 73.3% (11/15), yet cerebral lesions observed prenatally were much lower (46.7%). 85.7% (6/7) VUS without nervous findings had normal phenotype as far as we followed up. Postnatal brain damage and neurological symptoms onset in only one infant without MRI findings in utero. As to the group with NVI, multiple CRs were found in 31.2% (5/16) and 87.5% (14/16) had normal nervous imaging. In 2 NVI fetuses (p36, p59), cortical nodules proved by MRI supported the diagnosis of TSC clinically. Both of them underwent TOP, and the heart tissues testing confirmed the NVI result (data was not shown). In terms of the mutation genes, TSC2 occupied a dominant position. Considered the TSC2 pathogenic variants are related to a more severe phenotype than TSC1 [8], we compared them in fetal with CR(s). Both the frequency of TSC-causing mutations and numbers of CR had no significant difference (P > 0.05). However, TSC2 variants were more related to positive neuroimaging findings than TSC1 (P < 0.05). (Table 4)

Clinical outcome

Finally, 33 families decided to terminate the pregnancy. One of them (case 25) experienced intrauterine fetal death at GA of 26 weeks for severe hemodynamic alteration. A considerable tumor (28 mm × 28 mm) obstructed the left ventricular outflow tract and complicated with pericardial effusion/peritoneal effusion (Fig. 1c). Postnatal data were available for 23 neonates; four lost following up (Table 5).
Among the 23 newborns (15 males, 8 females), the tumor decreased or regressed in 15 of them (10 males, 5 females) no less than three months after birth. 9 of the 23 newborns were negative with TSC1/2 mutation, 7 with VUS, and 7 with LP. All the brain MRI positive fetus presented neurological symptoms within one year after birth. MRI imaging was standard in p37 and p61 before delivery, for whom carried a VUS and an LP mutation, respectively. While new SENs were found in both when epilepsy occurred at about half a year old. All the fetuses with LP variants were finally proved to have brain lesions excepted one (case 50). Case 50 showed a known TSC2 missense variant (c.1385G > A; p.R462H), which has already been classified as likely pathogenic for TSC (clinvar). NGS-trio analysis of the family confirmed the father also carried this mutation without TSC associated phenotype. Furthermore, the c.1385G > A genetic alteration was identified in the healthy sibling, aunt, and grandfather (Fig. 3a).

For case 33, it was the third time a fetal with CR has been pregnant by the phenotypically familiar parents. NGS-trio identified two de novo missense TSC2 mutations in the fetus (Fig. 3b). The couple decided to TOP immediately without further evaluation of the neurological examination. We had no more information to clarify the pathogenicity of the two mutants. Considering the uncertain significance of these variants and the possibility of gonadal mosaicism, the risk of another affected child being born remains existed.

Work-flow suggestion

Thinking over that mostly intrauterine CRs are first observed coincidentally through III level ultrasound during 22–26 GA except for the one who has detailed family history, we proposed a simplified clinical work-flow for this situation based on our experience. When cardiac tumors are detected in mid-trimester, a detailed fetal echocardiography examination is recommended firstly to assess the cardiac structure and function and to give proper treatment. Then, MRI for the nervous system is needed to be performed as well as the family history collection. For the CRs fetus whose family history ambiguous or mutation unknown, targeted NGS-Trios could be the most effective and time-saving path to search the molecular etiology. Genetic counseling should be provided after a comprehensive analysis of clinical symptoms, imaging, and molecular results, which is essential for pregnancy decision or clinical management. Follow up before and after birth are both indispensables. Benefit from the multidisciplinary approach, 68.9% fetuses (42/61) with CRs could get precise clinical/genetic diagnosis, or had known outcome, turned to be potentially clinically actionable.

Discussion

When cardiac rhabdomyomas are detected in a fetus, both the perinatal outcome dominated by tumor space-occupying and the long-term prognosis related to TSC are critical, but tricky. We try to assess perinatal risk and prenatal diagnosis of TSC with CR as the initial symptom through a multidisciplinary approach, which opens up the possibility of relatively reliable assessment for numerous TSC VUS.

The influence of tumor on perinatal outcome
The influence of space-occupying is the determinant of the perinatal outcome. Outflow tract obstruction is not rare occurred in approximately 10% of our subjects. But severe hemodynamics disorders were scattered partly because multiple CRs, which accounted for 70% in this study, tended to be smaller and chamber located.

Although 83.7% of tumors increased by the stimulation of maternal estrogen prenatally, the increment reduced after the 32nd week. So the masses were at their most significant in the mid/last trimester for which required more rigorous and careful monitoring. The earliest ultrasonic observable time of CR was reported at 15 weeks of GA, but the exact time of tumorigenesis is difficult to deduce. Case 6 provided some clues for early events in tumor formation. We conjectured that early-onset CRs might be manifested as diffuse ventricular hyperechoic and could affect the normal remodeling process in the heart. Thus, the diagnosis of TSC in some complex cardiac structural malformation fetuses might be underestimated or ignored. Although the proliferation of CRs seems self-limiting and independent of TSC gene alteration, the tumorigenesis might result in gene instability, such as a loss of heterozygosity or a second variaous. Supporting evidence comes from targeted-deep sequencing in TSC-associated hamartoma samples, roughly 2/3 of hamartomas from TSC individuals harbored two TSC hits. We tested CR tissues in two aborted TSC fetuses, and both the mutations were consistent with their amniotic fluid results (data was not shown), suggested a germline origin of mutations. It reported that the total mutational burden of TSC lesions was low and suggested a low mitotic index, which consistent with the slow-growing nature. Anyway, strengthen echocardiography monitoring and reassess tumors in middle pregnancy is indispensable.

Some postpartum study reveals that up to 23% children with CRs may present arrhythmia, which may be related to abnormal conduction of electrical excitement in tumor tissues near the atrioventricular junction. Tetsuya et al. reviewed 20 fetuses with CRs and speculated that, regardless of number and location, tumor diameter > 30 mm is associated with postnatal arrhythmia. We observed no arrhythmia in all the subjects including the one who suffered a single huge tumor located in LV exceeded 30 mm.

**The influence of tumor-related TSC on long-term prognosis**

The influence of CR-related TSC is a decisive factor for long-term prognosis although it is really a hot potato. NGS facilitates the detection process but leave the problem to variants recognition. We amplified the detection yield to 73.8% (including mutations of P, LP and VUS), which was much higher than ten years before. Chen, et al had identified TSC1/TSC2 P/LP variants in 69.8% fetus CR which seemed more instructive. We speculated it was a selection bias for inclusion criteria: over 90% of the fetal CR included in our study were accidental findings during ultrasound screening in phenotypically normal or presumed normal individual. Although the proportion of multiple CRs here was 10% less than Chen’ paper, both of our study considered that the correlation between CR and TSC is strong regardless of the presence of single or multiple tumors. Rhabdomyomas could not be fully confirmed pathologically until fetus tissues were obtained. Therefore, some other benign cardiac tumors, such as teratomas, fibroids, hemangiomas, or hamartomas, might pulled down the variants detection rate. In order to avoid this
selection bias as much as possible, we differentiate these tumors by rigorous fetal echocardiogram selection according to various characteristics before inclusion. Teratoma mostly presents as a single mixed mass in the pericardial cavity, often with pericardial effusion. Fibroid and hemangioma were often higher in size and grew fast, with or without calcification. Myxomas may attach to the atria and swing with the rhythm; also, malignant tumors are rare.

*Family phenotype collection* is in the top priority of genetic counseling. About 32% of mutations identified in this cohort were inherited from parents without typical phenotype. Clinical expression is hugely variable. Some subtle signs, mostly the skin manifestations, were easily overlooked or misdiagnosed until 3–6 outpatient visits to get a preliminary diagnosis in China. A couple who claimed to be “totally healthy” also need double check and confirmation of suspicious phenotype. p20 and p23 had both suffered from “acne” for decades, which was actually the atypical facial angiofibromas for TSC. (Fig. 3de). Mosaicism is another essential factor affecting phenotype, especially in mild cases with NVI by conventional testing, which according for 7.5% of patients with a clinical TSC diagnosis. Variant allele fraction (VAF) in the blood positively correlated with the number of major features. Interestingly, low-level mosaicism (0–10%, median 1.7%), which likely arising from a later postzygotic variant, had a milder and distinct clinical phenotype in comparison with other TSC series, with similar facial angiofibromas (92%) and kidney angiomyolipomas (83%) [23]. VAF of the p61 husband was 12%, he showed an unnoticeable asymmetric facial angiofibroma (Fig. 3a). However, for p61 infant who carried the heterozygous variant, not only facial lesions but also moderate mental retardation and epilepsy presented at 6 months. The difference in performance is not only closely related to the mosaic level, but may also be affected by factors such as the structure of the variant, the tissue distribution and the penetrance rate. Cutaneous signs are the most common signs in TSC phenotype series, vice versa, the variant of *TSC1/TSC2* was the most frequently to detect in skin lesions [24]. Thus, dermatological consultations or even skin biopsy sequencing are necessary for asymptomatic parents whose variant are accidentally discovered by NGS-trio to confirm the diagnosis, especially when encountering a VUS. The facial phenotype is a useful message for mosaic TSC, and may be the only clue for pathogenicity assessment with an uncertain variation.

Penetrance is high but not absolutely, which is also influences the assessment of pathogenicity of VUS. The *TSC2* R462H substitution has been recorded in individuals affected with TSC by Leiden Open-source Variation Database (http://www.LOVD.nl/) and been classified as likely pathogenic. In vitro experimental studies shown that this missense change has an effect on TORC1 activity and interferes with the formation of *TSC1-TSC2* complex, and results in an unstable protein and impairs protein function [25]. This mutant is not recorded in population databases (rs45494392, ExAC no frequency) but uniquely presented in fetal 50 and his/her clinically normal sibling, father, and grandfather. The fetal was negative for neuroimaging findings during the whole pregnancy. All index points to benign prognosis, which had been confirmed in following up. Integrated with genetic information in trios, and imaging results may enable early implementation of further diagnostic investigations, perinatal surveillance and family counseling, especially for couples with no signs of TSC phenotype on clinical examination.
For post-test analysis in the NGS era, it was common for phenotypic fetuses to show reported/novel VUS or NVI despite over 10000 variants that have been recorded in these two genes. Numerous VUS intensified the pathogenesis uncertainty and anxiety in pregnant women. Some other angles of view needed to be introduced to aid further evaluation. Pathological changes in the CNS are observed in almost all TSC individuals, and approximately 80–90% of them present cortical tubers and/or SENs, and seems equally familiar in \textit{TSC1} and \textit{TSC2} pathogenic mutation [24]. However, MRI is not a routine inspection. Neurological manifestations would be easily overlooked and mostly be checked after CR detection during the second-trimester scanning when the pathological changes in CNS might have gone a long way. Saada J et al. [13] indicate that the characteristic cerebral lesions of TSC may form as early as 10–20 weeks of embryonic development. As soon as 2007, Mühler MR et al. had preliminary explored the application of fetal cerebral MRI in sonographically proven CR [26]. Here, we disclosed that neuroimaging finding is a much more specificity indication of genetically TSC and was proportional to poor prognosis. \textit{TSC2} mutations seemed more likely to have neuropathy than \textit{TSC1 in prenatal}. Combination with fetal MRI, neurological lesions were found in 2 NVI cases and 5 \textit{TSC2} VUS cases, for whom clinically diagnosis of TSC was made. Exceptionally, in one case with VUS, neurological symptoms occurred, and brain damage originated four months after birth. Among the seven newborns with VUS, 2 (28.6%) developed epilepsy and had neuroimaging findings with following-up.

Benefit from prenatal MRI scanning, some VUS, or even NVI turned to be potentially clinically actionable. But sometimes, lesions might be later onset and are too tiny to find at the time of examination, wherefore any possible sign of occupation requires careful attention. p38 was identified as a de novo \textit{TSC2 LP} mutation, in 35 GA, only right ventriculomegaly had been sawing, but SENs progressed soon in infancy. Both case37 and case 61 identified mutation of \textit{TSC2} and had normal brain MRI at 24 GA and 22GA, respectively, while occurred epilepsy and observed SENs afterbirth. It was impossible to know the exact time of the occurrence of SENs as only brain MRI scanning was performed during the whole pregnancy. So, regular brain MRI monitoring cannot be ignored when the CR fetuses are carrying a P/LP/VUS mutation of \textit{TSC} genes.

It had been recognized that high-yield mutation detection methods would also help to reduce uncertainty and anxiety in the significant proportion of individuals and families for whom existing diagnostic methods are not informative. In a series of 38 TSC NVI individuals, 2 (6%) mosaic mutations and 5 (13%) heterozygous mutations that had been missed by other mutation detection methods were identified using exon-specific ultra-deep sequencing [21]. As epigenetic silencing having been demonstrated rare in \textit{TSC1} and \textit{TSC2} [15], possible other reasons for the inability to detect mutations in TSC NVI individuals was: gonadal chimerism, low-level somatic mosaicism, mutations hiding in deep intronic and regulatory regions or mutations to other as yet unidentified genes may cause TSC. For this consideration, the residual risk of TSC in the NVI subset was approximately 5–10% varies between platform [21].

Besides, the possibility of parental gonadal mosaicism cannot be debarred in a typical CR fetus with more than one proband sibling. The reported incidence of germ-line mosaicism range from 2–5% [27].
Case 33 here was a family had had two fetal with CR(s). For this pregnancy, two missense variations of \textit{TSC2} were detected but absent in both parents. Although both of the mutations were pathogenic ambiguous, it was highly suspected germ-line mosaicism in the couple. It implied that the recurrence risk of unaffected parents who have had an affected child would increase because of germline mosaicism, and families with high suspicion of this causing should be advised to prenatal diagnosis for each pregnancy.

**Conclusion**

We recommended strengthening the fetal heart examination during the middle and late pregnancy, especially for pregnancy with a family history of TSC. Fetal echocardiography is the preferred method of detecting fetuses CR. At the same time, neuroimaging evidence seems more effective than the number of CR to imply the pathogenicity of the mutation and a poor prognosis. Combined with neuroMRI and genetic testing, it facilitates early detection of TSC and is of great significance for perinatal management and prognostic guidance. The multidisciplinary approach could allow nearly 70% fetuses with CR(s) turning to be potentially clinically actionable.

**Limitation**

Our study has a few limitations that warrant further consideration. First, the pathological information of these cardiac tumors is neither sufficient for lacking an autopsy. For prenatal diagnosis of CR, symptom collection should be improved: hypomelanotic macules may be present prenatally, and skin exam could provide supporting evidence for diagnosis. Second, a limited number of cases identified \textit{TSC1} mutations, making it difficult to assess the relationship between CR, genotype, and neuroimaging changes for this subpopulation of TSC. Third, the clinical manifestations of TSC can be mimicked by mutations in many genes. Sequence coverage was restricted to exons and adjacent intronic, which perhaps missed mutations deep within introns or in other unknown regulatory regions.

**Abbreviations**

- CR: cardiac rhabdomyoma; TSC: tuberous sclerosis complex; MRI: Magnetic Resonance Imaging; VUS: variations of uncertain significance; P: pathogenic; LP: likely pathogenic; SENs: subependymal nodules; CNVs: copy number variations (CNVs); TOP: Termination of pregnancy; NVI: no variant identified; NGS: next generation sequence

**Declarations**

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Authors’ contributions

All authors have materially participated in the study and manuscript preparation. Y Qi carried out all the data analyses, participated in the design of the work and wrote the draft; H Ding and Y Zhang collected all clinical data, Y Huang, Y Zeng, L Yu and L Liu participated in molecular genetic test work; A Yin designed the work and revised the manuscript. All authors critically reviewed the manuscript and provided final approval for submission. All authors agree to be accountable for all aspects of the work, ensuring the accuracy and integrity of the publication.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Ethics Committee of the Guangdong Women and Children Hospital, Guangzhou, China (Number 201601046, approved on 01-22-2016).

Consent for publication

Obtained verbal consent for publication from the two pregnant women. They didn’t mind reporting these genetic information to research, and they also signed a written consent form.

Competing interests

The authors declare that they have no competing interest.

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Tables

Due to technical limitations, table 1-5 is only available as a download in the Supplemental Files section.