Single giant mediastinal rhabdomyoma as a sole manifestation of TSC in foetus

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Background. Presence of multiple cardiac rhabdomyomas is one of the major features of Tuberous sclerosis (TSC), but isolated progressing single giant rhabdomyoma is very rare and not typical of TSC.

Case report. This report presents family without obvious history of TSC with occurrence of giant mediastinal rhabdomyoma affecting the haemodynamics in male foetus, without other TSC symptoms. Girl from the next gravidity had prenatally detected multiple rhabdomyomas and small subcortical tuber of brain detected after birth. DNA analysis found novel c.4861A>T *TSC2* variant and large deletion in *TSC2* in tumour tissue from male foetus. The novel *TSC2* variant was also present in the girl and her healthy father, *in silico* analysis suggested its functional effect on *TSC2*. Brain MRI of the father detected mild TSC specific abnormality.

Conclusion. We suggest the novel *TSC2* mutation is a cause of mild TSC in this family and has reduced expression. The clinical and molecular findings in this family also emphasize that TSC diagnosis should be also evaluated in case of single giant foetal cardiac rhabdomyoma.

Key words: cardiac rhabdomyoma, *TSC2*, reduced expression, mild tuberous sclerosis.

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INTRODUCTION

The rhabdomyomas are the most common type of cardiac tumours in fetuses, newborns and children¹. Presence of multiple cardiac rhabdomyomas is one of the major features of tuberous sclerosis (TSC) and they can often be detected in the prenatal period. In contrast, single rhabdomyoma is not typical of TSC and giant mediastinal / cardiac tumour is a very rare finding in TSC (ref.^{2,3}). Here we report on a family without history of TSC with a huge mediastinal tumour in one foetus, multiple cardiac rhabdomyomas of foetus in the next gravidity and a novel *TSC2* variant found in 3 members of the family.

CASE REPORT

The foetus from the second gravidity showed increased nuchal translucence (NT) in the first trimester but the karyotype was 46,XY. During the USG follow up, there was a progression in NT thickness and echocardiography performed in 17+5 g.h. revealed the presence of a probable rhabdomyoma measuring 5 x 6 mm close to the mitral valve and tendency to bradycardia. According to the USG evaluation in the cardiologic centre, the finding

was not typical of rhabdomyomatosis in the 21st week of pregnancy. There was presence of pathological structures heading from the area of dorsal mediastinum and around heart atria, infiltrating the atrial walls and growing into the atrium. The mass was filling approximately one half of the right atrium cavity. A dense large structure (measuring 10 x 13 x 14 mm) was present in the pericardium with pericardial effusion. Due to the poor prognosis for the foetus, the parents opted for termination of the pregnancy in the 22nd week of gravidity. Autopsy described a male foetus without signs of external malformation. Chest organs were in common positions but there was a bulging mass under the pericardium. The tumour measured 16 x 12 x 8 mm, was on the surface of the left atrium and partly on the left ventricle surface and was tightly connected with the myocardium. A small amount of clear pleural fluid was present. Histology of the tumour confirmed presence of cardiac rhabdomyoma. Other organs were without signs of malformation.

In the next gravidity, slight hyperechogenicity of the mitral valve and posterior wall of the left heart ventricle was detected in the 26th week of pregnancy. Subsequent USG follow-up detected intracardial structures resembling rhabdomyomas in the left atrium and left ventricle in 31+2 g.h. The rhabdomyomas did not impair heart haemodynamic and had a non-progressive character.

Table 1. Result of *in silico* analysis of novel TSC2 mutation functional effect.

Online predictor	Result of <i>in silico</i> analysis
SIFT, PROVEAN	damaging, deleterious (for all of the assessed protein isoforms)
PolyPhen2	probably damaging, MSA*: Ile1621 is conserved in mammals
Mutationassessor	medium functional impact
Mutation Taster	disease causing, PhyloP score: 3.362

* MSA – multiple sequence alignment

Fig. 1. The sequenograms demonstrate heterozygous presence of c.4861A>T *TSC2* mutation (NM_000548.4, ENST00000219476) in father (F) and proband (P), and wild type sequence in paternal grandmother (GM). There is homozygous constitution of the mutation in DNA isolated from cardiac rhabdomyoma of foetus (FR) from second gravidity of proband's mother – the mutation actually affects only one allele, the second one is affected by deletion (according to the MLPA analysis).

Spontaneous delivery occurred in the 39th week of pregnancy; the girl had no obvious respiratory or cardiac troubles. Neonatal echocardiography described multiple rhabdomyomas without haemodynamic significance.

The family was referred to the genetic department when the girl was 6 weeks old; she showed no obvious TSC symptoms, psychomotor development was normal. Brain MRI examination at 9 weeks of life showed the presence of a tiny hypersignal lesion measuring 2.5 mm in the borderline of subcortical and centrum ovale regions of left frontal lobe – presumed to be a cortical/subcortical tuber. During the MRI follow up (at 1 year and 5 months and at 2 years and 6 months) the left frontal tuber showed no expansive character and another thin stripped lesion was uncovered just next to the body of the right lateral ventricle. The parents and older sister had no obvious TSC specific skin lesions or dental pits.

Results of genetic analysis

The Sanger sequencing of all coding regions in *TSC1* and *TSC2* was done with the informed consent of the parents - the unknown c.4861A>T / p.Ile1621Phe muta-

tion in 38th exon of *TSC2* (Fig. 1) was found in the proband. The mutation was present in the father, but absent in older sister, mother and grandmothers. In the next step, we assessed the effect of the mutation on *TSC2* protein using available online predictors. All of the five *in silico* tools predicted the *TSC2* mutation has a functional impact (Table 1). And more, the mutant allele was neither found in ExAC nor The thousand genomes databases. The absence of the variant in the databases also supports the functional impact of the novel *TSC2* mutation.

DNA analysis found the presence of the novel *TSC2* mutation in foetus (Fig. 1). The *TSC2* is a tumour suppressor and therefore, should the novel *TSC2* mutation have functional impact, then the cardiac tumour should contain second (somatic) mutation. Tumour and non-tumour tissues in foetal heart tissue (FFPE sample) were separated by a microdissection instrument and DNA was isolated. MLPA analysis of foetal cardiac rhabdomyoma tissue found the presence of exons 3-42 deletion in *TSC2* that was absent in normal myocardial tissue (Fig. 2). This finding also supported the functional impact of the novel *TSC2* mutation.

Fig. 2. The ratio chart displays results of MLPA analysis of *TSC2* (P046-C1 *TSC2*, Lot C1-0315, MRC Holland), analysis was performed by Coffalyser software. Analysed DNA was isolated from cardiac rhabdomyoma tissue (foetus from second pregnancy of proband's mother). In the case of clear deletion, signal of probe (red dots) lies below the red line. There is a deletion of exons 4-42 of *TSC2* and at least exon 46 of *PKD1*. Partially reduced signal of some probes (the exons 7, 8, 10, 15, 20, 21, 30, 31 and 42 of *TSC2*, purple dots) represent heterogeneity of cardiac tissue – tumour cells could not be entirely separated from cells of normal heart tissue. This finding demonstrates presence of the second hit (loss of heterozygosity) in *TSC2* in foetal cardiac rhabdomyoma. The red straight line in the bottom of the picture represents schematic extent of *TSC2*/*PKD1* deletion.

TSC shows variable expressivity but the penetrance should be complete, therefore we searched for other signs of *TSC* in the father. Although his abdominal USG scan did not find any pathology of the kidney, the brain MRI revealed the presence of fine hyperintensive streaks, suggestive of subcortical tubers, in the frontotemporal region. In T2 sequences, there were tiny hyperintensive nodes (not larger than 2 mm) in the caudothalamic region. The supratentorial changes described on brain MRI were therefore consistent with diagnosis of *TSC*.

DISCUSSION

The p.Ile1621Phe mutation is located in GAP domain of *TSC2* that is crucial for inactivating Rheb (Rheb activates mTORC1 complex). Several causal mutations were described in this region. Hoozeveen-Westerveld et al.⁴ performed functional assessment of several *TSC2* variants, including variants surrounding the p.Ile1621Phe mutation. They proved that p.His1620Arg and p.Thr1623Ile mutations were less effective at inhibiting mTORC1, these mutations showed significantly higher mean T389/S6K ratio than wild-type *TSC2*. The ratio was not different from causal p.Arg611Gln mutation.

The proband and her father also have rs13335638 polymorphism (intron 37). Neither this polymorphism nor the c.4861A>T *TSC2* mutation was detected in the

paternal grandmother. This indicates the mutation should originate from the paternal grandfather - he had renal disease from his twenties, underwent two surgeries of the kidney and died of end stage renal disease at the age of 42. Unfortunately, his medical records are not available; therefore possible *TSC* symptoms related to kidney disease could not be excluded. The mutation could, however, be of de novo origin in the father and the kidney problems in the paternal grandfather may have been caused by an unrelated process. Interestingly, the paternal origin of *TSC2* mutation in a family with very mild symptoms of *TSC* was also assumed in a report by Mayer et al.⁵

TSC shows variable severity of symptoms, there is both interfamilial and intrafamilial variability of phenotype expression. Women usually tend to have milder disease than men.

The sporadic *TSC2* mutations are linked with more severe phenotype of *TSC* as has been observed in cases without a family history of *TSC*. In cases of familial *TSC2* mutations, the phenotype is less severe⁶ and there are some reports of mildly affected *TSC* families also presenting with prenatally detected multiple cardiac rhabdomyomas^{5,7}. Multiple foetal / neonatal cardiac rhabdomyomas were significantly associated with *TSC*; they can be found in up to 95 % cases of *TSC* (ref.^{8,9}). In contrast, the occurrence of single intracardiac rhabdomyoma is not over-represented in persons with *TSC* compared to non *TSC* patients² and single giant progressing rhabdomyoma is

very rare in persons with TSC. Schlaegel et al.¹⁰ described a case similar to our family – a foetus showing a large mediastinal tumour with pleural effusion and mediastinal shift at 27 weeks of gravidity and negative TSC focused family history. The tumour increased in size during the USG follow up. A second smaller intracardiac tumour appeared at 36 weeks of pregnancy. The cardiac findings were confirmed in the postnatal period, rhabdomyomas were without haemodynamic compromise. The CNS pathology was uncovered only postnatally, the neurological examination of neonate had normal findings. Genetic analysis confirmed TSC. During the follow up USG pathology of kidney was not detected and the cardiac tumours showed spontaneous regression (MRI examination showed the presence of only one rhabdomyoma measuring 33 mm).

Some large cardiac rhabdomyomas could require surgical intervention (because of haemodynamic compromise), but oral administration of everolimus (mTOR inhibitor) may be also considered¹¹. CNS pathology may be detected prenatally; therefore brain MRI examination of foetuses with cardiac rhabdomyomas is recommended¹². The mTOR inhibitors are now used to treat a variety of TSC-associated symptoms. Moreover, there is also evidence of successful treatment of brain pathologies by rapamycin in the prenatal period in well established mouse TSC models^{13,14}. Although these results allow for the potential of prenatal mTOR inhibitors administration to treat large cardiac rhabdomyomas that are seriously affecting haemodynamic, more research is needed prior to the treatment of human foetuses with TSC – mice treated prenatally with rapamycin showed some level of cognitive function impairment¹³.

CONCLUSION

Cardiac rhabdomyomas tend to regress/diminish in a high proportion of TSC cases within the first few years of life. This is also the case for our proband. She is now almost 4 years old and her cardiac rhabdomyomas are regressing. Although rare, the cardiac rhabdomyoma could appear again, therefore regular follow up by a cardiologist is reasonable for some time (at least until the onset of puberty) for all TSC patients¹⁵. With reference to the family described in our report, we suggest that TSC should be also considered in any case of single giant mediastinal rhabdomyoma regardless of TSC focused family history.

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REFERENCES

1. Carrilho MC, Tonni G, Araujo Júnior E. Fetal cardiac tumours: prenatal diagnosis and outcomes. *Rev Bras Cir Cardiovasc* 2015;30(1):VI-VII.
2. Verhaaren HA, Vanakker O, De Wolf D, Suys B, François K, Matthys D. Left ventricular outflow obstruction in rhabdomyoma of infancy: meta-analysis of the literature. *J Pediatr* 2003;143(2):258-63.
3. Lee KA, Won HS, Shim JY, Lee PR, Kim A. Molecular genetic, cardiac and neurodevelopmental findings in cases of prenatally diagnosed rhabdomyoma associated with tuberous sclerosis complex. *Ultrasound Obstet Gynecol* 2013;41:306-11.
4. Hoogeveen-Westerveld M, Ekong R, Povey S, Mayer K, Lannoy N, Elmslie F, Bebin M, Dies K, Thompson C, Sparagana SP, Davies P, van Eeghen AM, Thiele EA, van den Ouweland A, Halley D, Nellist M. Functional assessment of TSC2 variants identified in individuals with tuberous sclerosis complex. *Hum Mutat* 2013;34:167-75.
5. Mayer K, Goedbloed M, van Zijl K, Nellist M, Rott HD. Characterisation of a novel TSC2 missense mutation in the GAP related domain associated with minimal clinical manifestations of tuberous sclerosis. *J Med Genet* 2004;41:e64.
6. Sancak O, Nellist M, Goedbloed M, Elferich P, Wouters C, Maat-Kievit A, Zonnenberg B, Verhoef S, Halley D, van den Ouweland A. Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: genotype-phenotype correlations and comparison of diagnostic DNA techniques in Tuberous Sclerosis Complex. *Eur J Hum Genet* 2005;13:731-41.
7. Chen CP, Su YN, Hung CC, Shih JC, Wang W. Novel mutation in the TSC2 gene associated with prenatally diagnosed cardiac rhabdomyomas and cerebral tuberous sclerosis. *J Formos Med Assoc* 2006;105:599-603.
8. Chao AS, Chao A, Wang TH, Chang YC, Chang YL, Hsieh CC, Lien R, Su WJ. Outcome of antenatally diagnosed cardiac rhabdomyoma: case series and a meta-analysis. *Ultrasound Obstet Gynecol* 2008;31:289-95.
9. Tworetzky W, McElhinney DB, Margossian R, Moon-Grady AJ, Sallee D, Goldmuntz E, van der Velde ME, Silverman NH, Allan LD. Association between cardiac tumours and tuberous sclerosis in the foetus and neonate. *Am J Cardiol* 2003;92:487-9.
10. Schlaegel F, Takacs Z, Solomayer EF, Abdul-Kaliq H, Meyberg-Solomayer G. Prenatal diagnosis of giant cardiac rhabdomyoma with fetal hydrops in tuberous sclerosis. *J Prenat Med* 2013;7:39-41.
11. Ilczoch E, Hanslik A, Luckner D, Kitzmüller E, Prayer D, Michel-Behnke I. Prenatal diagnosis of giant cardiac rhabdomyoma in tuberous sclerosis complex: a new therapeutic option with everolimus. *Ultrasound Obstet Gynecol* 2015;45(5):618-21.
12. Mühler MR, Rake A, Schwabe M, Schmidt S, Kivelitz D, Chaoui R, Hamm B. Value of fetal cerebral MRI in sonographically proven cardiac rhabdomyoma. *Pediatr Radiol* 2007;37:467-74.
13. Way SW, Rozas NS, Wu HC, McKenna J 3rd, Reith RM, Hashmi SS, Dash PK, Gambello MJ. The differential effects of prenatal and/or postnatal rapamycin on neurodevelopmental defects and cognition in a neuroglial mouse model of tuberous sclerosis complex. *Hum Mol Genet* 2012;21(14):3226-36.
14. Tsai PT, Greene-Colozzi E, Goto J, Anderl S, Kwiatkowski DJ, Sahin M. Prenatal rapamycin results in early and late behavioral abnormalities in wildtype C57BL/6 mice. *Behav Genet* 2013;43(1):51-9.
15. Thatte NM, Guleserian KJ, Veeram Reddy SR. New-onset cardiac rhabdomyoma beyond infancy in a patient with tuberous sclerosis complex. *Cardiol Young* 2016;26:396-9.