CASE REPORT

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Novel Intronic Heterozygous Mutation in TSC2 Gene in Pediatric Patient with Tuberous Sclerosis Complex

ABSTRACT

Background: Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder and involves multiple organs, intellectual disability and epilepsy. Mutations in TSC1 and TSC2 genes are responsible for the molecular disease mechanism. **Objective:** The aim is to determine molecular background of a patient with a suspicion of TSC. **Case presentation:** In this case report, we describe a seven year old patient with the clinical manifestation of TSC that includes supratentorial changes, subependymal hamartomas and angifibromas in the facial area. Besides the brain and skin changes, no other TSC characteristics were observed. The patient was referred to molecular genetic testing using Next Generation Sequencing (NGS). Results: Clinical exome sequencing revealed intronic TSC2 c.4849+2T>G variant. The variant was confirmed using Sanger sequencing on the subject. However, the variant was not detected in the parents, which indicated that it arose de-novo. The RegSNP-intron, Mutation Taster and Human Splicing Finder were used as a bioinformatic tools to predict the possible effect on protein. Using bioinformatic tools, it was determined that the variant is possibly damaging to protein. **Conclusion:** This data suggest that observed splicing intronic variant could be the cause of TSC in this pediatric patient.

Keywords: Tuberous Sclerosis Complex, TSC2, intronic variants, Next Generation Sequencing.

1. BACKGROUND

Tuberous sclerosis complex (TSC) is a multisystem disorder and involves multiple organs (heart, skin, kidneys, brain and lungs) (1). It is an autosomal dominant progressive disorder which is characterized by the occurrence of multiple tumors in different organs, epilepsy, intellectual disability and facial angiofibromas. Because of the different locations of the lesions, clinical manifestation of the disease can highly vary among individuals and the major cause of mortality are renal complications and seizures (2, 3). The disorder is caused by the mutations in TSC1 and TSC2 genes, which are tumor suppressor genes that encode hamartin and tuberin, and are located on chromosomes 9q34.3 and 16p13.3, respectively. The encoded proteins interact and form heterotrimeric complex, called the TSC protein complex (4, 5). The most widely accepted molecular disease mechanism is that TSC1/TSC2 mutations inactivate the TSC protein complex, which leads to loss of inhibitory effects on the mTOR (mammalian target of rapamycin) pathway (6, 7). The phenotypic expression of the disease and symptoms are highly variable, and it can be difficult to establish clinical diagnosis. It is established that even monozygotic twins may have different clinical manifestations of the disease (8). Molecular testing has proven itself to be important additional tool for TSC diagnosis, especially using Next Generation sequencing (NGS).

2. OBJECTIVE

Here, we present the clinical data and manifestation of a pediatric patient with TSC and detection of novel intronic TSC2 c.4849+2T>G variant detected by NGS. Confirmatory Sanger sequencing of the target region on the subject and both of his parents was conducted.

3. CASE PRESENTATION

A 6-month-old infant presents for examination due to episodes of afebrile convulsions. The patient is the second child from the regular, gynecologically controlled pregnancy. Due to the fact that the mother has epilepsy verified at the age of 15, the pregnancy is carried out by a planned caesarean section. After the birth, the newborn cried immediately, he was not resuscitated. As part of early psychomotor development, it is reported that the patient spoke his first words at 7-8 months, walked at 16 months. Ultrasound of the brain showed regular circular hyperechoic shadows - intensity of calcifications. EEG registration in spontaneous sleep showed increased electrocortical epileptic activity of generalized hypsarrhythmia type. Next generation sequencing panel for epilepsy was negative. CT of the neurocranium showed supratentorial bilateral frontotempoparietal, para and supraventricular, juxta and subcortical visible confluent hypodense zones that are calcified frontally and frontobasally, more to the left. Subependymal multiple formations were visible in the lateral cerebral ventricles, which are mostly calcified. MRI of the neurocranium showed supratentorial changes according to the type of cortical/subcortical tubercles and subependymal hamartomas and thickened cortex in terms of cortical dysplasia (Figure 1 and 2).

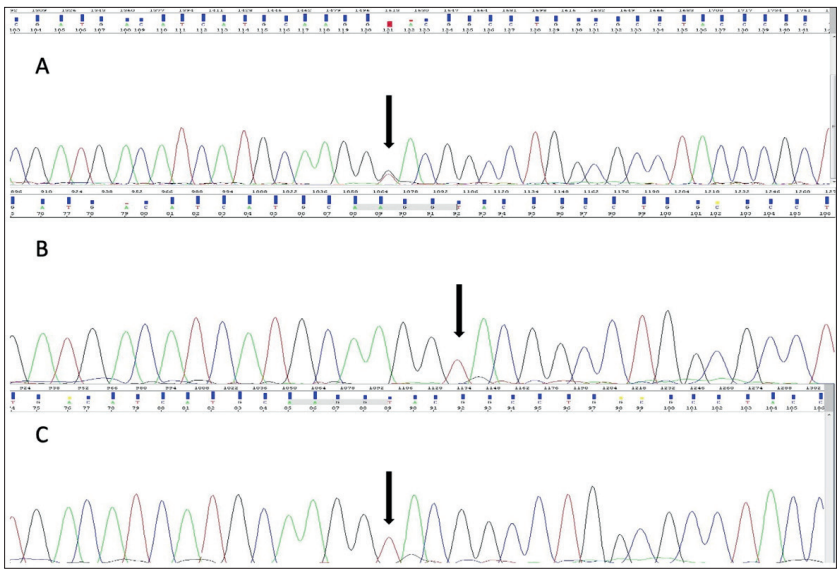

Figure 1. SAG T2W frontoparietal cystic formation with MRI signs of radial band favoring Tuberous Sclerosis Complex

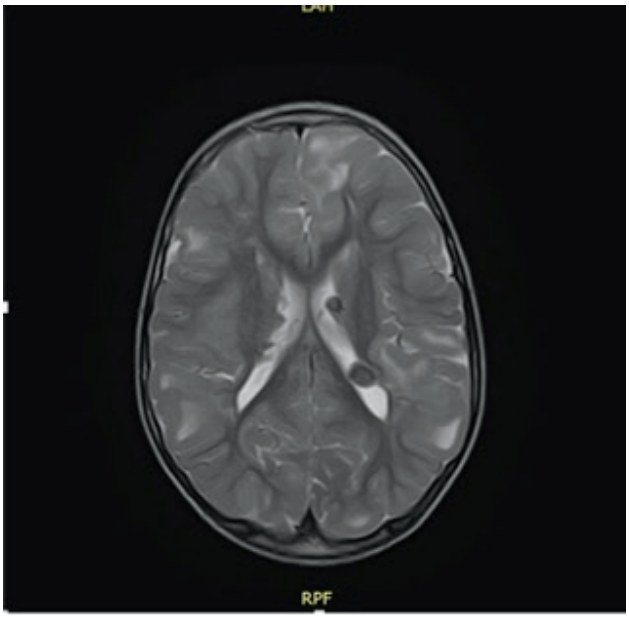

Figure 2. Subependymal hamartroma in the area of the left lateral ventricle

Due to the clinical manifestation of West's syndrome, vigabatrin is prescribed in the therapy, with which a good control of epileptic seizures is obtained. After initial treatment and suspicion of TSC, the patient is under the multidisciplinary supervision of a pediatric neurologist, nephrologist, cardiologist, psychologist and radiologist. Nephrological, cardiological and ultrasound diagnostics of the abdomen did not show any organ changes within the mentioned organ systems. Psychological assessment showed indicators that point to disharmonious development, especially in the area of language-speech functions, social communication and focus of attention. The patient was hospitalized several times due to febrile convulsions. Re-evaluation at the age of 4.5 showed skin changes in the form of hypopigmentation on the skin of the neck, right hand, left leg, angiofibromas in the facial area and swelling in the projection of the left scapula. The aforementioned change was surgically removed in 2024 (PH finding: Nuchal type fibroma). With the exception of skin and brain changes, no other major or minor characteristics of TSC are recorded. During continued follow-up, there was no progression of the MRI findings on the brain.

Seven year old patient has been referred to comprehensive genetic testing in 2024 due to suspicion of West syndrome and TSC, with history of multiple seizures. After obtaining informed consents from the parents, DNA was extracted from buccal swab sample using Qiagen DNA Mini kit (Qiagen, Hilden, Germany) according to manufacturers instructions. Library preparation was performed using Illumina Trusight One panel (Illumina, San Diego, California, USA) that covers over 4500 genes. DNA library preparation consists of tagmentation of genomic DNA, amplification of tagmented DNA, clean up of amplified DNA, probe hybridization, library pooling, second hybridization, clean up of captured libraries, clean up of enriched libraries and finally libraries quantification and qualification using DNA Bioanalyzer Agilent 2100 (Agilent, Santa Clara, California, USA). Sequencing was perfomed on Illumina NextSeq 550 Dx (Illumina, San Diego, California, USA) instrument. FastQ files were obtained and quality control metrics, as well as variant calling was done using Varsome software (Saphetor, Lausanne, Switzerland).

Clinical exome sequencing revealed TSC2 c.4849+2T>G heterozygous intronic mutation, not previously described in ClinVar, but LOVD's prediction of the mutation was pathogenic. Sanger sequencing was performed as a confirmational

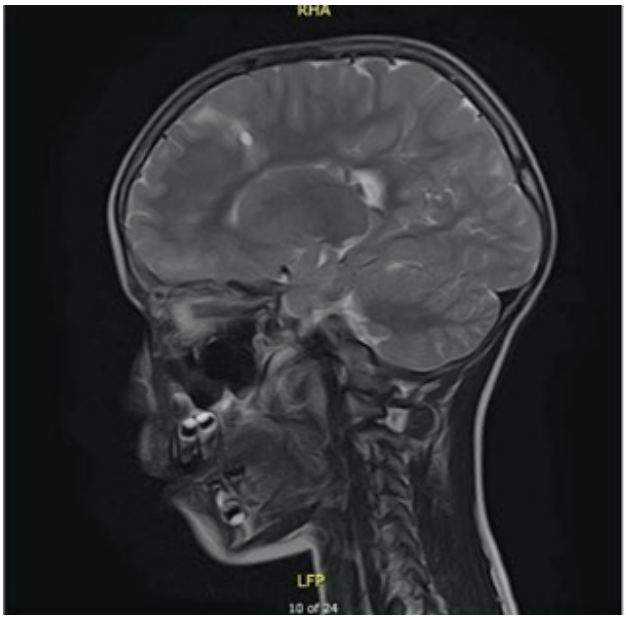

Figure 3. Electropherogram displaying heterozygous variant on TSC2 gene on proband (A), homozygous wild type on parent 1 (B) and homozygous wild type on parent 2 (C)

method for pediatric patient and his parents to determine whether the variant arose de novo. Primers were designed using Primer3 software according to results obtained using clinical exome sequencing. Primer sequences are as follows: forward primer 5' CCAGCCGGACAAGGTGTA 3' and reverse primer 5' CTCCCATCCAGTCCTGCTAC 3'. After amplification, gel electrophoresis was performed to confirm the presence of amplification products. Amplified products were then purified using ExoSap (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and Brilliant Dye (NimaGen, Nijmegen, Nethelands) was used for cycle sequencing. Products purification was done using NucleoSpin columns (MACHEREY-NAGEL, Düren, Germany) and products were sequenced on Seqstudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sanger sequencing results confirmed the heterozygous mutation on a pediatric patient, while his parents are wild type for the certain variant (Figure 3).

The RegSNP-intron and Mutation Taster bioinformatic tools were used to predict the disease-causing probability of detected intronic variant, which is on splicing site. The probability of this intronic variant to be disease-causing is 65% (reference values 45-100%), according to RegSNP-intron. These results indicate that the heterozygous intronic variant on TSC2 gene is the cause of the disease in this patient. Human Splicing Finder tool was additionaly used to predict variant effects on mRNA splicing. Transcript ENST00000219476 was used using HGVS nomenclature and the results showed alteration of the wild type donor site affecting splicing, with reduced score of the wild type donor site from 89.54 to 62.4.

4. DISCUSSION

Published clinical studies describe a large heterogeneity of clinical presentation (9-11) within TSC. One-third to one-half of patients with a confirmed diagnosis of TSC are expected to develop a pharmacologically resistant form of epilepsy with the appearance of their first epileptic seizures in infancy (12). Our patient was clinically diagnosed with TSC at the age of 6 months. On prescribed first-line therapy, patients have been without evident convulsive attacks for the last four years, with the expected cognitive delay. Considering the individuality of the clinical symptoms of each individual patient, at this moment the current condition is observed, and in case of worsening of the disease in the form of exacerbation of pharmacologically resistant epileptic attacks, further modalities of therapy include the treatment of the attacks themselves and the treatment of SEG tumors by prescribing mTOR inhibitors, specific formulations of CBD and VNS (13).

Genetic testing is imperative in TSC diagnostics, due to varying symptoms (14). Clinical exome sequencing was performed on a pediatric patient due to the suspicion of West syndrome and TSC. In silico analysis predicted that the obtained c.4849+2T>G variant is on splicing site and with high damage probability. Ye & Zeng (2019) have sequenced the whole exome of a four generation Chinese TSC family with five affected and five unaffected individuals. The researchers identified intronic TSC2 heterozygous variant in affected individuals. Their in- silico analysis suggest that the variant leads to frame-shift and premature stop codon in the protein. It has been identified that most splicing abnormalities occur in small truncating mutations in the TSC2 gene, in comparison to TSC1 gene (15).

Confirmational Sanger sequencing was performed on proband and his biological parents. Subject's parents are wild type for the obtained variant. Tyburczy et al. (2015) have performed NGS on 53 subjects with TSC and they have identified mutations in 45 subjects. Splicing site mutations were identified in 40% of the cases. Most of these mutations were found in sporadic TSC cases, not detected in parental samples (16).

It is important to note that 10-25% clinically diagnosed TSC patients have no detected mutations on TSC1 or TSC2 genes, due to the mutations in intronic or promoter regions, lower sensitivity molecular testing, etc (17). Resolving the issue of NMIs (No Mutation Identified) patients with TSC would not be possible without the usage of NGS technologies, due to the coverage of whole length of TSC1 and TSC2 genes, including intronic sequences (18).

Based on bioinformatic predictions, novel mutation described in this paper affects splicing, due to the alteration of the donor site. Observed variant is located at the +2 position of the directly upstream exon. It is estimated that splice site mutations account for approximately 15% of all disease-causing small mutations (19). According to Xu et al. (2000), the most severe effect on trans-splicing has +2 intronic position (20). Sequence changes that cause splicing defects are most frequently found at the donor and acceptor sites at positions +1, +2 and -1, -2, respectively (15).

5. CONCLUSION

This case report represents novel TSC2 intronic heterozygous c.4849+2 T>G variant in 7 year old patient with the suspicion of West syndrome and TSC detected with clinical exome panel. The subject was referred to genetic testing because of one major and one minor clinical manifestation of TSC. Confirmatory Sanger sequencing was performed on the subject and both his parents and it was confirmed that the mu-

tation arose de-novo. This case report suggests that intronic variants can be responsible for the clinical manifestations of TSC.

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- **Conflict of interest:** The authors have no conflict of interest to declare.
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REFERENCES

1. Qiu C, Li C, Tong X, Dai L, Liu W, Xie Y, Zhang Q, Yang G, Li T. A novel TSC1 frameshift mutation c.1550_1551del causes tuberous sclerosis complex by aberrant splicing and nonsense-mediated mRNA degradation (NMD) simultaneously in a Chinese family. Mol Genet Genomic Med. 2020; 8(10): e1410.
2. Northrup H, Wheless JW, Bertin TK, Lewis RA. Variability of expression in tuberous sclerosis. J Med Genet . 1993; 30: 41–43.
3. Zöllner JP, Franz DN, Hertzberg C, Nabbout R, Rosenow F, Sauter M. A systematic review on the burden of illness in individuals with tuberous sclerosis complex (TSC). Orphanet J Rare Dis. 2020; 15: 23.
4. Kwiatkowski DJ, Manning BD. Molecular basis of giant cells in tuberous sclerosis complex. N Engl J Med. 2014; 371(8): 778–780.
5. Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. Trends Cell Biol. 2015; 25(9): 545–555.
6. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. N Engl J Med. 2006; 355(13): 1345–1356.
7. Lipton JO, Sahin M. The neurology of mTOR. Neuron. 2014; 84(2): 275-291.
8. Humphrey A, Higgins JN, Yates JR, Bolton PF. Monozygotic twins with tuberous sclerosis discordant for the severity of developmental deficits. Neurology. 2004; 62: 795–798.
9. Dzefi-Tettey K, Edzie EK, Gorleku P, Piersson AD, Cudjoe O. Tuberous sclerosis: A case report and review of the literature. Cureus. 2021; 13(1): e12481.
10. Alshoabi SA, Hamid AM, Alhazmi FH, Qurashi AA, Abdulaal OM, Aloufi KM, Daqqaq TS. Diagnostic features of tuberous sclerosis complex: case report and literature review. Quant Imaging Med Surg. 2022; 12(1): 846-861.
11. Frost M, Hulbert J. Clinical management of tuberous sclerosis complex over the lifetime of a patient. Pediatric Health Med Ther. 2015; 6: 139-146.
12. Miszewska D, Sugalska M, Jóźwiak S. Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review. J Clin Med. 2021; 10(23): 5495.
13. Northrup H, Aronow ME, Bebin EM, Bissler J, Darling TN, de Vries PJ. International Tuberous Sclerosis Complex Consensus Group. Updated International Tuberous Sclerosis Complex Diagnostic Criteria and Surveillance and Management Recommendations. Pediatr Neurol. 2021; 123: 50-66.
14. Ye Y, Zeng Y. Whole exome sequencing identifies a novel intron heterozygous mutation in TSC2 responsible for tuberous sclerosis complex. Sci Rep. 2019; 9(1): 4456.
15. Mayer K, Ballhausen W, Leistner W, Rott H. Three novel types of splicing aberrations in the tuberous sclerosis TSC2 gene caused by mutations apart from splice consensus sequences. Biochim Biophys Acta. 2000; 1502(3): 495-507.
16. Tyburczy ME, Dies KA, Glass J, Camposano S, Chekaluk Y, Thorner AR, Lin L, Krueger D, Franz DN, Thiele EA, Sahin M, Kwiatkowski DJ. Mosaic and Intronic Mutations in TSC1/TSC2 Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing. PLoS Genet. 2015; 11(11): e1005637.
17. Krueger DA, Northrup H. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol. 2013; 49: 255–265.
18. Avgeris S, Fostira F, Vagena A, Ninios Y, Delimitsou A, Vodicka R, Vrtel R, Youroukos S, Stravopodis DJ, Vlassi M, Astrinidis A, Yannoukakos D, Voutsinas GE. Mutational analysis of TSC1 and TSC2 genes in Tuberous Sclerosis Complex patients from Greece. Sci Rep. 2017; 7(1): 16697.
19. Mayer K, Ballhausen W, Leistner W, Rott HD. Three novel types of splicing aberrations in the tuberous sclerosis TSC2 gene caused by mutations apart from splice consensus sequences. BBA. 2000; 1502(3): 495-507.
20. Xu Y, Liu L, Michaeli S. Functional analyses of positions across the 5' splice site of the trypanosomatid spliced leader RNA. Implications for base-pair interaction with U5 and U6 snRNAs. J Biol Chem. 2000; 275(36): 27883-92.