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Kristin M. Ikeda
London Health Sciences Centre

Andrew A. House
Schulich School of Medicine & Dentistry

Dervla M. Connaughton
Schulich School of Medicine & Dentistry

Stephen E. Pautler
Schulich School of Medicine & Dentistry

Victoria Mok Siu
Schulich School of Medicine & Dentistry, vmsiu@uwo.ca

See next page for additional authors

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Authors

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Potential Pitfalls in Pre-implantation Genetic Diagnosis in a Patient with Tuberous Sclerosis and Isolated Mosaicism for a *TSC2* Variant in Renal Tissue

Kristin M. Ikeda[a, b]   Andrew A. House[b, c]   Dervla M. Connaughton[b, c]
Stephen E. Pautler[b, d]   Victoria Mok Siu[b, e]   Michelle-Lee Jones[a, b]

[a]Department of Clinical Neurological Sciences, London Health Sciences Centre, London, ON, Canada;
[b]Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada; [c]Division of Nephrology, Department of Medicine, London Health Sciences Centre, London, ON, Canada; [d]Department of Surgery, London Health Sciences Centre, London, ON, Canada; [e]Division of Medical Genetics, Department of Pediatrics, London Health Sciences Centre, London, ON, Canada

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Established Facts

- Pathogenic inactivating variants in *TSC1* or *TSC2* are identified in 85% of the patients with tuberous sclerosis complex (TSC) using conventional sequencing methods.
- Mosaicism accounts for some cases of TSC where a germline mutation is not found.
- Tumours in TSC typically occur when there is a germline mutation in one allele followed by a "second hit" with somatic mutation in the other allele.
- Pre-implantation genetic diagnosis offers couples the opportunity to prevent transmission of genetic disease.

Novel Insights

- Detection of a mosaic variant in select tissues may not represent the primary pathological mutation if there are systemic manifestations.
- Caution is advised for pre-implantation genetic diagnosis in patients with mosaicism and limited tissue mutation expression.

Abstract
Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that displays a wide spectrum of clinical manifesta-
tions, often affecting multiple organs including the kidneys, brain, lungs, and skin. A pathogenic mutation in either the *TSC1* or *TSC2* gene can be detected in almost 85% of the cases, with mosaicism accounting for about half of the remaining cases. We report a case of TSC diagnosed clinically, requesting genetic counselling regarding reproductive risks. No mutation was identified on initial testing of peripheral

Dervla M.Connaughton
Division of Nephrology, Department of Medicine
London Health Sciences Centre
339 Windermere Road, PO Box 5339 London N6A5A5 (Canada)
dervla.connaughton@lhsc.on.ca

blood; however, mosaicism for a likely pathogenic frameshift variant in *TSC2* was detected at a level of 15% in renal angiomyolipoma tissue. Despite widespread clinical manifestations of TCS, this variant was not detected in skin fibroblasts or saliva, raising the possibility this is an isolated somatic mutation in renal tissue with the underlying germline mutation not yet identified. This case highlights the difficulties when counselling patients with mosaicism regarding their reproductive risks and prenatal diagnostic options.

Introduction

Tuberous sclerosis complex (TSC) is a complex autosomal dominant disorder characterized by growth of hamartomas in multiple organ systems [Northrup and Krueger, 2013]. It has an incidence of 1 in 6,000 live births and is associated with heterozygous pathogenic mutations involving either the *TSC1* or *TSC2* tumour suppressor genes, encoding hamartin and tuberin, respectively. About two-thirds of all cases are de novo, while one-third of the cases are inherited [Northrup et al., 1999].

Case Report

A 25-year-old female presented to the Genetics Clinic requesting information on prenatal diagnostic options related to tuberous sclerosis. She initially came to medical attention for right flank pain. Abdominal ultrasound and computed tomography (CT) scan showed bilateral renal angiomyolipomas (AMLs) (Fig. 1a, b), measuring 0.8 × 1.4 cm on the right and 7.8 × 7 × 13 cm on the left. There was no history of seizures or other neurological abnormalities other than migraine headaches. Cognitive function was excellent. There were no cardiac, pulmonary or visual complaints. Family history was negative for any relatives with TSC. A maternal aunt and one of her sons had learning disabilities, and another son died of a congenital heart defect.

Clinical examination revealed angiofibromas on both sides of her nose, and a fibrous cephalic plaque on the forehead, but no hypomelanotic macules, ungual fibromas, or shagreen patch. Enamel pits were found on dental exam.

Further screening demonstrated small retinal hamartomas in the right eye, multiple cortical tubers in the superior frontal gyrus, left occipital lobe, right fusiform gyrus and right upper Sylvian fissure, and a subependymal nodule (Fig. 1c, d). No lymphangioleiomyomatosis (LAM), cardiac rhabdomyomas, subependymal giant cell astrocytomas, or bony lesions were found. In total, she exhibited 5 major criteria and 1 minor criteria, satisfying the diagnostic criteria of 2 or more major findings for a clinical diagnosis of TSC [Verhoef et al., 1999; Northrup and Krueger, 2013].

The patient was started on sirolimus to slow the rate of growth of the AMLs, but she developed worsening migraine headaches and frequent skin rashes, which resolved in discontinuation of this treatment. She was subsequently started on everolimus, which was better tolerated, but AML growth continued, necessitating embolization of the largest renal AML. However, at follow-up 9 months later, the AML still measured 6.5 × 5.8 × 11.5 cm. A left robot assisted partial nephrectomy was performed approximately 1 year after initial presentation, at which time biopsies of affected renal tissue and overlying skin were obtained for DNA testing.

Molecular and Cytogenetic Investigations

Testing of DNA from peripheral blood was performed on 2 occasions using a combination of next-generation sequencing (NGS) and Sanger sequencing to cover the full coding regions of both *TSC1* (NM_000368.4) and *TSC2* (NM_000548.3). This included analysis of intronic regions within 20 bases of flanking each exon. This testing was performed through a commercially available panel at Prevention Genetics (www.preventiongenetics.com), and no pathogenic or likely pathogenic variants were found in DNA from peripheral blood.

MLPA was also performed using a multiplex PCR-based reaction. This individual was negative for deletions and duplications within the genomic region encompassing the *TSC1* (NM_000368.4) and *TSC2* (NM_000548.3) genomic regions. At the time, MLPA probe mixes used included: (1) P124-TSC1, which had full gene coverage of 1 probe per exon (exons 1–23), and (2) P046-TSC2, which had full gene coverage of 1 probe per exon (exons TSC-upstream-41, now known as exons 1–42).

Karyotyping was performed on peripheral blood to investigate for a translocation involving *TSC1* or *TSC2*, with normal results. Microarray analysis was performed using the Affymetrix CytoScan HD microarray. Genomic linear positions were given relative to NCBI genome build 37 (hg19), while analysis was performed using Chromosome Analysis Suite (ChAS; Affymetrix, version NA32.3). Chromosome microarray testing also yielded no CNVs involving *TSC1* or *TSC2*. Therefore, this patient was initially classified as TSC with no pathogenic variant identified. NGS on AML tumour tissue obtained at time of partial nephrectomy showed heterozygosity for a likely pathogenic variant (American College of Medical Genetics (ACMG) classification category 4) in NM_000548.3(*TSC2*):c.2046dup (p.Ser683Valfs*20) with mosaicism at a level of ~15%. The minimum acceptable coverage for all reported genomic regions by NGS was >25×. There were no regions reported with <25× read depth. In addition, as per Prevention Genetics protocol, any regions with insufficient coverage by NGS were covered by Sanger sequencing.

NGS on cultured fibroblasts obtained from a skin biopsy of the abdomen at the site of nephrectomy surgical incision yielded negative results for the c.2046dupG variant. A sample of DNA from saliva also did not reveal the variant.

Discussion

The *TSC2* c.2046dupG variant found in this patient's AML results in a frameshift and premature truncation of the protein (p.Ser683Valfs*20) which has not previously been described in the TSC Variation Databases (http://chromium.lovd.nl/LOVD2/TSC/home.php). The variant is predicted to be pathogenic, since the majority of
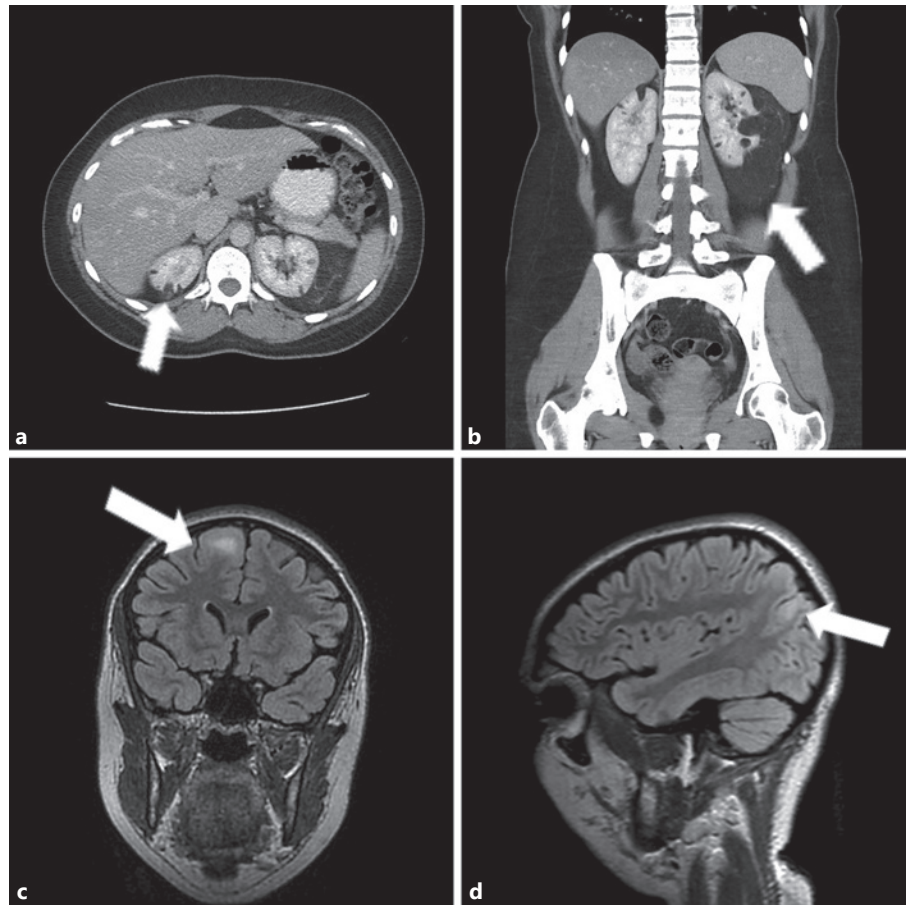
Fig. 1. Computed tomography imaging of abdomen and brain. **a** Axial image on CT abdomen – arrow indicates position of the right angiomyolipoma. **b** Coronal image on CT abdomen – arrow indicates position of the left angiomyolipoma. **c** CT brain – arrow indicates a cortical tuber in the superior frontal gyrus. **d** CT brain – arrow indicates a cortical tuber in the left occipital lobe.

TSC mutations are inactivating. Our patient did not have the *TSC2*: c.2046dupG variant or any other variant identified in skin, blood or saliva, despite the widespread clinical manifestations of TSC affecting brain (hamartomas), skin (angiofibromas and forehead plaque), eyes (retinal hamartomas), kidneys (AMLs), and teeth (enamel pits).

In patients with a clinical diagnosis of TSC, conventional genetic testing detects a mutation in about 85% of the cases, with one-third involving *TSC1* and two-thirds involving *TCS2*. Of those with no mutation identified, NGS detects mosaicism for mutations in blood, saliva, or AML in about half [Verhoef et al., 1999]. Five to ten percent of the cases carry a variant of uncertain significance or remain with no mutation identified [Peron et al., 2018]. In mosaic cases, skin biopsies tend to have a higher mutation detection rate than either saliva or blood, often with a higher mutant allele frequency compared to saliva or blood [Tyburczy et al., 2015a].

Crino et al. [2010] showed that 15 out of 16 individuals with germline *TSC1* or *TSC2* mutations had a somatic mutation in cortical tubers, supporting Knudson's 2-hit hypothesis of tumour development due to biallelic gene inactivation [Crino et al., 2010]. A study of facial angiofibromas demonstrated a *TSC1* or *TSC2* mutation, either germline or mosaic, in 82% of the patients, with a second hit demonstrated in half of these cases [Tyburczy et al., 2014]. In patients with germline *TSC2* mutations and multifocal renal cell carcinomas, multiple independent second hit point mutations or loss of heterozygosity have been identified in the renal tumours [Tyburczy et al., 2015b]. The combination of pulmonary LAM with renal AML constitutes 1 major criterion for the diagnosis of TSC, and may also occur sporadically [Carsillo et al., 2000]. Patients with TSC-associated LAM usually have 1 *TSC2* germline mutation and 1 acquired mutation in the affected tissues, while those with sporadic LAM have 2 somatic mutations [Smolarek et al., 1998; Carsillo et al., 2000]. Thus, in patients with germline TSC mutations, the finding of mosaicism for a different TSC variant in a TSC-related tumour usually constitutes the second hit.

There are 2 possible interpretations of the molecular test results in this case: (1) the patient is mosaic for the c.2046dupG variant throughout the body, but at a low level which was undetected in non-renal tissues, and the "second hit" in the AML was undetected, or (2) the patient has a germline variant which remains undetected with NGS, and the c.2046dupG variant is a somatic mutation present only in the sampled AML.

Since there was low-level mosaicism in tumour tissue but widespread clinical manifestation of TSC, we propose that the second explanation is more likely. There are however a number of limitations of using NGS methods which should be highlighted:

Firstly, minor sequence variants due to somatic mosaicism and sequence variants present in less than 50% of the patient's nucleated cells may also go undetected. We attempted to overcome this issue by performing analysis in a variety of cell-types.

Secondly, NGS may miss variants within runs of mononucleotide repeats (e.g., $(A)_n$ or $(T)_n$ with n > 8 in the reference sequence). These regions tend to be difficult to analyse because of strand slippage during amplification and therefore could be overlooked. Importantly, splice site mutations located more than 20 nucleotides away from the exon-intron boundary may be undetected. This is important since deep intronic variants can be detected in up to 40% of the patients with TSC [Tyburczy et al., 2015a]. Although not available in this case, future studies using whole-genome sequencing may detect additional variants within deep intronic sites, located greater than 20 nucleotides away from the exon-intron boundary.

Finally, NGS and Sanger sequencing often do not capture or amplify certain regions of the genome due to a deletion or insertion. For this reason, additional testing via MLPA was performed for the detection of CNVs, which was also negative.

Pre-implantation genetic diagnosis (PGD) is increasingly being used as a method to prevent transmission of genetic diseases in at-risk couples [Brezina and Kutteh, 2015]. However, PGD is only possible if the causative mutation has previously been identified in the family. When a prospective parent has a germline pathogenic variant, the chance of passing on the mutation is 50%. If there has been a post-zygotic mutation causing TSC in the prospective parent, the level of mosaicism can vary from tissue to tissue, and recurrence risk depends on the percentage of gonadal cells harbouring the mutation [Naja et al., 2016]. In males, it is sometimes possible to detect gonadal mosaicism through sperm analysis [Giannikou et al., 2019].

In this patient, if PGD were performed and the c.2046dupG were to be identified in an embryo, a diagnosis of TSC could be confidently made. However, since the variant likely represents the "second hit", isolated to the AML which was sampled, the probability of finding that variant at the time of PGD would be extremely low. If the c.2046dupG were absent at PGD, the possibility of TSC could not be ruled out, and might be as high as 50% if there is an undetected germline mutation. The same situation would apply to prenatal diagnosis with chorionic villus sampling or amniocentesis. The patient was counselled that PGD would be an expensive and unreliable option to achieve her goal of having an unaffected child. Egg donation would be the only option to ensure that any TSC variants present in this patient would not be passed on to any offspring.

This case outlines the challenges of providing genetic counselling with regards to utility of pre-implantation or prenatal diagnosis. Caution should be taken when counselling patients with no mutation identified and somatic mosaicism isolated to one tissue regarding their reproductive risks.

Statement of Ethics

Conflict of Interest Statement

Funding Sources

Author Contributions

References

Brezina PR, Kutteh WH. Clinical Applications of Preimplantation Genetic Testing. BMJ. 2015; 350:g7611.

Carsillo T, Astrinidis A, Henske EP. Mutations in the Tuberous Sclerosis Complex Gene TSC2 Are a Cause of Sporadic Pulmonary Lymphangioleiomyomatosis. Proc Natl Acad Sci USA. 2000;97(11):6085–90.

Crino PB, Aronica E, Baltuch G, Nathanson KL. Biallelic TSC gene inactivation in tuberous sclerosis complex. Neurology. 2010;74(21): 1716–23.

Giannikou K, Lasseter KD, Grevelink JM, Tyburczy ME, Dies KA, Zhu Z, et al. Low-level mosaicism in tuberous sclerosis complex: prevalence, clinical features, and risk of disease transmission. Genet Med. 2019;21(11):2639–43.

Naja RP, Dhanjal S, Doshi A, Serhal P, Delhanty J, SenGupta SB. The Impact of Mosaicism in Preimplantation Genetic Diagnosis (PGD): Approaches to PGD for Dominant Disorders in Couples Without Family History. Prenat Diagn. 2016;36(9):864–70.

Northrup H, Krueger DA. Tuberous Sclerosis Complex Diagnostic Criteria Update: Recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol. 2013;49(4):243–54.

Northrup H, Koenig M, Pearson D, Au K, Adam M, Ardinger H, et al. Tuberous Sclerosis Complex. GeneReviews. [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2021. Initial posting: July 13, 1999; last update: April 16, 2020.

Peron A, Au KS, Northrup H. Genetics, Genomics, and Genotype-Phenotype Correlations of TSC: Insights for Clinical Practice. Am J Med Genet C Semin Med Genet. 2018;178(3):281–90.

Smolarek TA, Wessner LL, McCormack FX, Mylet JC, Menon AG, Henske EP. Evidence That Lymphangiomyomatosis Is Caused by TSC2 Mutations: Chromosome 16p13 Loss of Heterozygosity in Angiomyolipomas and Lymph Nodes From Women With Lymphangiomyomatosis. Am J Hum Genet. 1998;62(4):810–5.

Tyburczy ME, Wang JA, Li S, Thangapazham R, Chekaluk Y, Moss J, et al. Sun exposure causes somatic second-hit mutations and angiofibroma development in tuberous sclerosis complex. Hum Mol Genet. 2014;23(8):2023–9.

Tyburczy ME, Dies KA, Glass J, Camposano S, Chekaluk Y, Thorner AR, et al. Mosaic and Intronic Mutations in TSC1/TSC2 Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing. PLoS Genet. 2015a;11(11):e1005637.

Tyburczy ME, Jozwiak S, Malinowska IA, Chekaluk Y, Pugh TJ, Wu CL, et al. A shower of second hit events as the cause of multifocal renal cell carcinoma in tuberous sclerosis complex. Hum Mol Genet. 2015b;24(7):1836–42.

Verhoef S, Bakker L, Tempelaars AM, Hesseling-Janssen AL, Mazurczak T, Jozwiak S, et al. High Rate of Mosaicism in Tuberous Sclerosis Complex. Am J Hum Genet. 1999;64(6): 1632–7.