Published in final edited form as:

Br J Dermatol. 2020 January ; 182(1): 235–237. doi:10.1111/bjd.18332.

A diagnostic algorithm for enhanced detection of mosaic tuberous sclerosis complex in adults

A.M. Treichel^{1,2}, D.J. Kwiatkowski³, J. Moss², T.N. Darling¹

¹Department of Dermatology, Uniformed Services University of the Health Sciences, Bethesda, MD

²Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

³Division of Pulmonary Medicine and of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Keywords

Tuberous sclerosis complex; mosaic; next-generation sequencing; genetics; no mutation identified; variant allele fraction

Dear Editor,

Tuberous sclerosis complex (TSC) is caused by pathogenic variants in *TSC1/TSC2*, resulting in the formation of hamartomas in multiple organs. The clinical diagnosis of TSC is typically made during childhood as most individuals develop epilepsy and have multiple clinical findings including those involving the skin^{1,2}. Some patients experience minimal morbidity throughout childhood but develop serious disease manifestations as adults.³ In those with subtle clinical findings, biopsy of TSC-related cutaneous tumours and genetic testing of the blood are often pursued⁴.

Routine genetic testing of blood DNA in children and adults with a clinical diagnosis of TSC fails to identify a pathogenic variant in about 15% of individuals⁵. In many of those with no mutation identified (NMI), the inability to identify a pathogenic variant is due to mosaicism and a variant allele fraction (VAF) below detection using Sanger sequencing.⁶ Our goal was to determine phenotypic indicators of individuals likely to be NMI with routine genetic testing, for whom next generation sequencing (NGS) of affected skin or other affected tissues may be considered. Utilising previously published data on our TSC

Corresponding Author: Dr. Thomas Darling, MD PhD, Thomas.Darling@usuhs.edu.

Conflicts of Interest:

The authors have no conflicts of interest to declare.

Disclaimer:

The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University, the Department of Defense or the National Institutes of Health.

This work was presented at the 2019 International Tuberous Sclerosis Complex Research Conference on June 20–22, 2019 in Toronto, Ontario, Canada.

cohort⁶ and the association of absent tubers and subependymal nodules (SENs) with NMI⁷, we propose a diagnostic algorithm to guide the genetic workup for adults with TSC.

Between 1998–2019, 119 adults diagnosed with TSC underwent evaluation at our institution. Forty-eight had genetic analysis or a family history of TSC and 71 had unknown genotypes^{6,8}. There were 29 individuals with germline and 19 with mosaic TSC, including 12 with symmetrically-distributed angiofibromas (AFs) and 7 with asymmetrically-distributed AFs, defined as $\geq 75\%$ of lesions on one side of the nose and cheeks⁶. In the current study, we used the 48 individuals with known germline or mosaic TSC⁶, and determined the positive predictive value (PPV) for germline or mosaic status, considering both the age of onset, and presence of eight mucocutaneous findings (MCFs) and five internal findings of TSC, distribution of facial AFs, and total number of MCFs. Findings with a PPV $>70\%$ for germline or mosaic disease were incorporated into a diagnostic algorithm, and then applied to the 71 individuals with TSC and unknown genotype (Figure 1).

Phenotypes suggestive of germline TSC included onset of ungual fibromas (UFs) before age 15 years (PPV=92%, 11/12 patients, CI: 0.61–1.0), AFs before age 5 years (77%, 10/13, 0.46–0.95), and the presence of ≥ 3 MCFs plus SENs (71%, 20/28, 0.51–0.87). Individuals with these phenotypes fell into the group consisting of mostly germline TSC and therefore molecular genetic evaluation should begin with routine testing of the blood DNA. If no pathogenic variant is identified, then NGS of the blood should be done next, as 4/5 mosaic individuals within this category had a blood VAF $>1\%$ (Figure 1).

Phenotypes suggestive of mosaicism included the presence of < 3 MCFs (PPV=88%, 7/8 patients, CI: 0.47–1.0), absence of tubers and SENs (100%, 3/3, 0.29–1.0), and asymmetrically distributed AFs with < 100 lesions (100%, 7/7, 0.59–1.0). Individuals with these phenotypes fell into the group consisting of mostly low-level mosaic TSC. The blood VAF of mosaic individuals within this group was $< 1\%$ in 6/9 individuals. In contrast, 15/19 of their skin tumour samples had a VAF $>1\%$ ⁶. Thus, the first step of their genetic workup should include NGS of TSC-related tumours. Once a pathogenic variant is identified, targeted sequencing methods such as amplicon NGS can be used to assess variant prevalence in other tissue (blood, urine, saliva, normal skin, skin tumour, or internal tumour), to confirm the variant as mosaic rather than a second hit somatic mutation.

We applied our diagnostic algorithm to 71 individuals with sporadic TSC and unknown genotypes. This included 6 who had routine genetic testing of the blood, all of whom were NMI. The algorithm sorted 44 into the germline group, 6 into the possible mosaic group, and 15 into the low-level mosaic group (excluding 6 with insufficient clinical information). Individuals who were NMI were more frequent in the low-level mosaicism group (6/15, 40%) than in the germline group (0/44, 0%) (Fisher Exact $p=0.0001$). Although these individuals with NMI did not undergo analysis for mosaicism, this independent validation analysis suggests that our clinical algorithm correctly identifies those individuals with TSC who are likely to fail routine blood DNA analysis for TSC gene mutations.

In conclusion, we propose a stepwise clinical algorithm applicable to adults with TSC that provides guidance for an efficient approach to TSC gene pathogenic variant identification. This algorithm identifies those most likely to have mosaicism, and if implemented can accelerate variant identification and counseling.

Acknowledgements:

Written informed consent was obtained according to IRB-approved protocols 00-H-0051, 95-H-0186 96-H-0100, and/or 82-H-0032.

Funding Sources:

Research reported in this publication was supported in part by the Intramural Research Program, National Institutes of Health (NIH), National Heart, Lung, and Blood Institute (NHLBI); the NIH, National Institute of Arthritis and Musculoskeletal and Skin Diseases, under Award Number R01AR062080; NIH, NHLBI under Award Number 1U01HL131022; the Doris Duke Charitable Foundation Clinical Research Mentorship grants #2018042; the Tuberous Sclerosis Alliance, Engles Fund for Research in TSC and LAM. Additionally, this work was made possible through the NIH Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and generous contributions to the Foundation for the NIH from the Doris Duke Charitable Foundation, the American Association for Dental Research, the Colgate-Palmolive Company, Genentech, and other private donors. For a complete list, visit the foundation website at <http://www.fnih.org>.

References:

1. Northrup H, Krueger DA, International Tuberous Sclerosis Complex Consensus G. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol*. 2013;49(4):243–254. [PubMed: 24053982]
2. Teng JM, Cowen EW, Wataya-Kaneda M, et al. Dermatologic and dental aspects of the 2012 International Tuberous Sclerosis Complex Consensus Statements. *JAMA Dermatol*. 2014;150(10):1095–1101. [PubMed: 25029267]
3. Seibert D, Hong CH, Takeuchi F, et al. Recognition of tuberous sclerosis in adult women: delayed presentation with life-threatening consequences. *Ann Intern Med*. 2011;154(12):806–813, W-294. [PubMed: 21690595]
4. Nathan N, Burke K, Moss J, Darling TN. A diagnostic and management algorithm for individuals with an isolated skin finding suggestive of tuberous sclerosis complex. *Br J Dermatol*. 2017;176(1):220–223. [PubMed: 27167640]
5. Tyburczy ME, Dies KA, Glass J, et al. 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. [PubMed: 26540169]
6. Treichel AM, Hamieh L, Nathan NR, et al. Phenotypic distinctions between mosaic forms of tuberous sclerosis complex. *Genet Med*. 2019.
7. Boronat S, Shaaya EA, Doherty CM, Caruso P, Thiele EA. Tuberous sclerosis complex without tubers and subependymal nodules: a phenotype-genotype study. *Clin Genet*. 2014;86(2):149–154. [PubMed: 23909698]
8. Tyburczy ME, Wang JA, Li S, et al. Sun exposure causes somatic second-hit mutations and angiofibroma development in tuberous sclerosis complex. *Hum Mol Genet*. 2014;23(8):2023–2029. [PubMed: 24271014]

Figure 1: A diagnostic algorithm for the clinical detection of individuals with TSC likely to have mosaicism, and recommended genetic testing.

Phenotypic features were used to predict individuals with 1) germline TSC, 2) possible mosaic TSC, and 3) low-level mosaic TSC. The relative proportion of germline and mosaic individuals within each group are represented by blue and green, respectively. For those in the first group, genetic workup should begin with routine genetic testing of blood or saliva DNA. Individuals in the second group should begin with NGS of the blood or saliva DNA. The third group should begin with NGS of a skin tumour biopsy in order to enhance the detection of mosaic pathogenic variants. Abbreviations: AF, angiofibroma; UF, ungual fibroma; SEN, subependymal nodule; TSC, tuberous sclerosis complex; NGS, next-generation sequencing; NMI, no mutation identified; VAF, variant allele fraction.

^a Asymmetrical distribution of facial angiofibromas is defined as ≥ 75% on one side of the nose and cheeks in an individual with fewer than 100 lesions.

b Mucocutaneous findings were counted if present in sufficient number to fulfill the diagnostic criteria and included the following eight features: AF (3), hypomelanotic macule (3, at least 5 mm diameter), fibrous cephalic plaque, shagreen patch, UF (2), dental pitting (3), oral fibroma (2), and confetti skin lesions (hypomelanotic macules ranging from 1–5 mm in diameter).

c Exon-based Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis for pathogenic variants in *TSC1* and *TSC2*.

d Next generation sequencing with analysis for pathogenic variants at low allele fractions. *e* Simultaneously obtain additional tissue samples (blood, urine, saliva, normal skin, skin tumour, internal tumour) for targeted or amplicon NGS to detect the pathogenic variant identified in the skin tumour in order to confirm mosaicism.

f Alternatives should be considered for those who remain NMI after NGS. This includes consideration for alternative diagnoses (germline or mosaic Birt-Hogg-Dubé or MEN1) and undetectable *TSC1* or *TSC2* variants (due to deletion, intronic mutation, extreme low-level mosaicism). Approaches to enhance detection of mosaicism may include sequencing of internal tumour, skin tumour fibroblast culture, or analysis of skin tumour dermis to enrich for clonal fibroblast populations.