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Low-Level Mosaicism in Tuberous Sclerosis Complex (TSC): Diagnostic and Clinical Implications From Two Novel Cases and Literature Review

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ABSTRACT

Mosaicism is relatively common in Tuberous Sclerosis Complex (TSC) but can be difficult to detect using routine diagnostic tests, particularly when the variant allele frequency (VAF) is low. We describe two cases of mosaic TSC diagnosed using an ultra-deep sequencing approach in multiple tissues and review the literature about this topic in order to discuss new diagnostic paradigms. In the first case, further testing was prompted by the presence of angiomyolipomas in the otherwise unaffected 51-year-old father of a woman diagnosed with TSC2; the familial pathogenic variant was present with a very low VAF in angiomyolipoma tissue and peripheral blood. The second case, a 17-year-old boy diagnosed with infantile myofibromatosis, presented dermatological and brain MRI findings suggestive of TSC; a *TSC1* pathogenic variant was first identified on DNA extracted from angiofibroma biopsy, and then confirmed on non-lesional skin, peripheral blood, and saliva. The identification of the causative *TSC1/2* variant is crucial to provide appropriate management and genetic counseling for family planning. Most mosaic individuals in the literature have cutaneous features of TSC; in the presence of an accessible lesion, we recommend considering a tissue biopsy to have a higher chance of identifying a low-level mosaicism.

1 | Introduction

Mosaicism in Mendelian disorders is probably more common than previously thought, particularly when present at a low variant allele frequency (VAF), as this makes identification difficult with standard diagnostic approaches. This phenomenon is particularly frequent in many conditions caused by alterations in genes involved in the mTOR signaling pathway, as well as in tumor suppressor genes and in genes with a high spontaneous mutational rate. The *TSC1* and *TSC2* genes, associated with Tuberous Sclerosis Complex (TSC), belong to all three categories, and indeed mosaicism is known to be relatively common in TSC patients (Rose et al. 1999; Verhoef et al. 1999).

It has been shown that all types of *TSC1/2* variants may present at a mosaic status, and a higher representation of splice-site variants has been observed in mosaic compared to homogeneous variant carriers (Tyburczy et al. 2015). The mosaic variants can occur on the allele derived from either parent, and both gonadal mosaicism and gonadosomatic mosaicism have been reported

The first two authors contributed equally to this study.

(Martínez-Glez et al. 2020; Wang et al. 2021). Furthermore, these variants may occur at very low VAFs (< 1%–5%), meaning they are likely to be missed on routine diagnostic testing, particularly if sequencing is performed exclusively on DNA extracted from peripheral blood, where the variant may be absent (Giannikou et al. 2019).

In mosaic individuals, the phenotype severity and the number of organs involved appear to be partly dependent on the timing of post-zygotic mutation, which is reflected in the VAF value in different tissues (Giannikou et al. 2019; Nathan et al. 2017; Treichel et al. 2019). However, even very low VAFs (< 5%) can be associated with severe phenotypes that meet the clinical diagnostic criteria for TSC (Byers et al. 2018; Manzanilla-Romero et al. 2021), and other elements, such as the involvement of *TSC1* versus *TSC2*, as well as the specific *TSC1/2* variant, must be considered when evaluating possible genotype–phenotype correlations. In general, the presence of less than three mucocutaneous findings, the absence of tubers and subependimal nodules, and an asymmetric distribution of angiofibromas with less than 100 lesions have been found to be suggestive of mosaicism.

Around 10%–15% of individuals who meet the clinical criteria for TSC test negative on conventional *TSC1* and *TSC2* analyses, which include DNA sequencing and deletion/duplication analysis; mosaicism is believed to explain up to 50%–60% of those cases, while the remaining quota may be explained by variants within introns or regulatory regions (not covered by standard NGS protocols) or by technical issues (Tyburczy et al. 2015).

Strategies to improve the diagnostic rate for TSC include the use of deep sequencing technologies (Qin et al. 2010), and the use of multiple tissues for molecular testing. Selecting the most suitable tissue for analysis (which ideally should be relatively accessible and have a high VAF) represents a significant challenge, as VAFs appear to be highly variable in different individuals (Ye et al. 2022). In general, VAF is higher in a skin lesion biopsy compared to unaffected skin tissue, blood, or saliva (Giannikou et al. 2019; Tyburczy et al. 2015); shagreen patches and ungual fibromas have the highest VAFs, while limited information is available regarding the VAF in less accessible tissues such as angiomyolipomas (Giannikou et al. 2019; Tyburczy et al. 2015).

In this perspective, we describe two cases of TSC mosaicism where the molecular diagnosis had important implications, and we review the literature on this topic to try to define the most effective diagnostic strategy to apply to these patients.

2 | Materials and Methods

2.1 | Patient Recruitment and Clinical Assessment

The two patients described in this study were assessed and recruited by the Nephrogenetics and Dermatology outpatient services, respectively, both of which are integrated within the IRCSS Azienda Ospedaliero Universitaria of Bologna. The patients had provided written informed consent to the use of their biological samples and data for research purposes. Ethical review and approval were waived for this study because, according to the local policy, informed consent is considered sufficient for reports of an observational nature concerning a limited number of patients.

2.2 | Molecular Analyses

An ultra-deep targeted next-generation sequencing (NGS) approach for hereditary cancer genes using IonGene Studio S5 System, including *TSC1* and *TSC2*, was performed at the Medical Genetics Unit laboratories of the IRCCS Azienda Ospedaliero-Universitaria of Bologna in all index cases, with the aim of detecting causative variants and identifying potential mosaicism. The analysis was conducted on multiple tissue types in both cases. DNA was extracted from peripheral blood and angiomyolipoma tissue in the first patient, and from peripheral blood, saliva, skin lesion, and unaffected skin in the second. The list of genes included in the NGS panel is provided on request. Genomic coordinates are based on the Genome Reference Consortium Human Build 19 (GRCh37/hg19).

Raw sequencing data were transferred to the Torrent Server, where the Torrent Suite software performed alignment to the reference genome and generated FASTQ files, Binary Alignment Map (BAM) files, Binary Alignment Index (BAI) files, and Variant Call Format (VCF) files. All VCF files were uploaded into the Ion Reporter software (Thermo Fisher Scientific Inc., Waltham, MA, USA), using the Annotation Variant workflow to annotate each variant with nucleotide changes in the mRNA transcript, corresponding amino acid substitutions, exon or intron locations, and predicted functional effects. BAM/BAI files generated from the alignment were visualized using the Integrative Genomics Viewer (IGV) software to assess sequencing read depth, zygosity, read quality, and mapping quality.

Large *TSC1*/*TSC2* rearrangements were excluded using multiplex ligation-dependent probe amplification (MLPA).

The presence of the variant identified through NGS was confirmed by Sanger sequencing in all sampled tissues.

2.3 | Literature Review

Previously reported cases of TSC mosaicism were identified through a literature search in PubMed (latest access: June 2025). Only studies describing at least one individual with a confirmed mosaic pathogenic/likely pathogenic variant were included; reports of "suspected mosaicism" lacking molecular confirmation were excluded. Additionally, studies reporting a single case with a mosaic pathogenic or likely pathogenic *TSC1/2* variant identified exclusively in one tissue were excluded due to the potential that such variants represent a "second hit" rather than the primary pathogenic variant. Articles providing molecular data without accompanying clinical information were also excluded. To avoid duplication, data from individuals reported in multiple studies was considered only once.

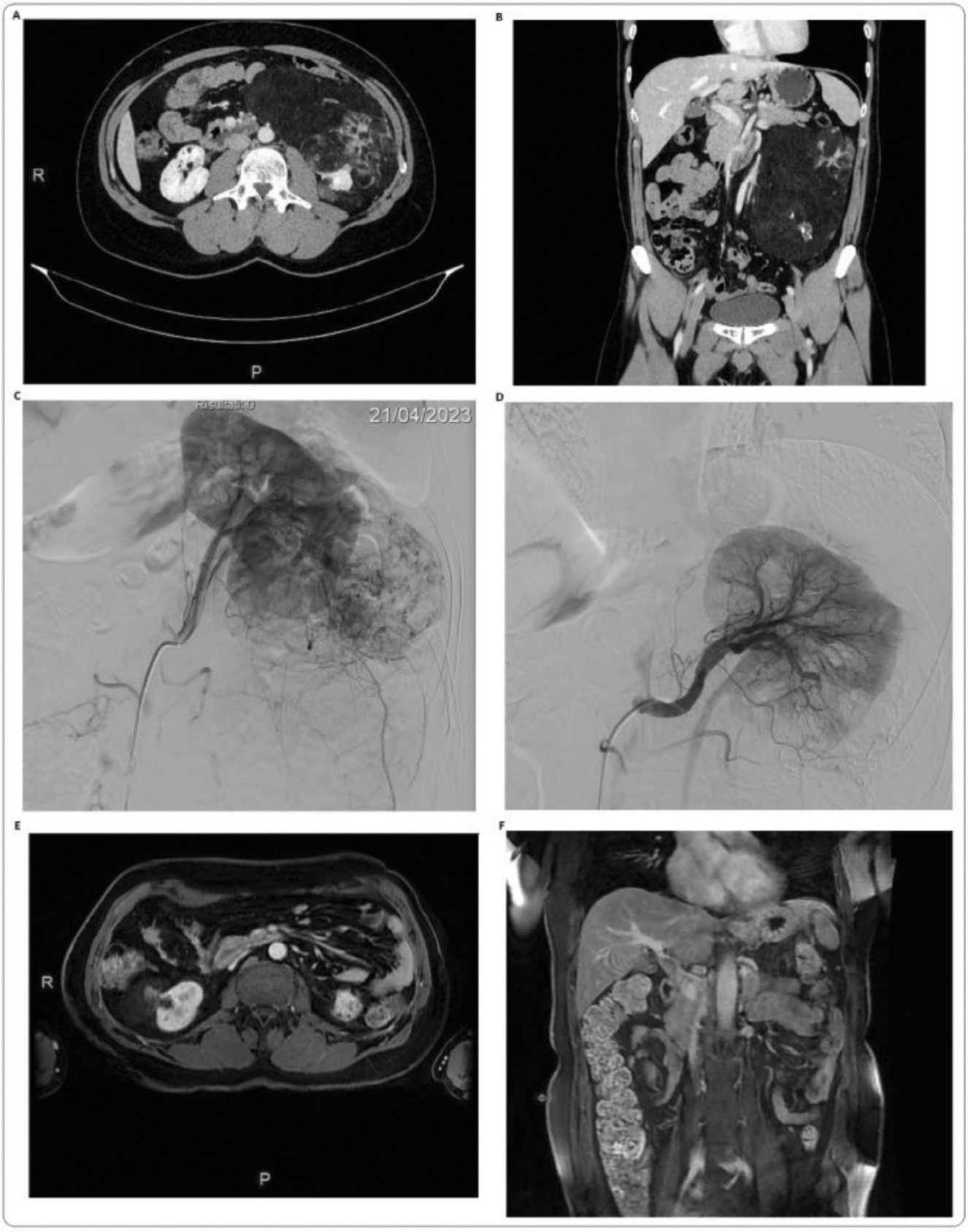

FIGURE 1 | CT scans showing an angiomyolipoma in the left kidney displacing abdominal organs to the right (A, B). Angiography confirming a hypervascular lesion before embolization (C) and loss of vascularization after treatment (D). Follow-up MRI showing a significant reduction in size of the mass and improvement in the displacement of abdominal structures (E, F).

We collected aggregated information on clinical features (paying particular attention to the TSC major diagnostic criteria), on the diagnostic tests that led to the identification of a pathogenic variant, and on the different samples analyzed.

3 | Results

3.1 | Case Reports

3.1.1 | Case 1

A 51-year-old man came to our attention while accompanying his 21-year-old daughter to an appointment at the Nephrogenetics Clinic of our institute. The daughter, who was diagnosed with TSC in infancy, had intellectual disability, epilepsy with seizure onset at 7 months, cortical tubers on brain MRI, a history of small cardiac rhabdomyomas, hypochromic skin lesions, retinal hamartomas, and angiomyolipomas of the kidneys, which were the main indication for the follow-up appointment. Her diagnosis had been confirmed via Denaturing High Performance Liquid Chromatography (DHPLC) and Sanger sequencing analysis, which identified the heterozygous *TSC2*:c.3397+2T>G pathogenic variant that alters a canonical splice site and is predicted to disrupt splicing via the loss of the donor site (Splice predictor MobiDetails) (Baux et al. 2021).

At that time, segregation analysis was performed in both parents and in the proband's twin brother via DHPLC; the variant was not found in the family members and appeared to be de novo (Figure 1C).

However, anamnestic data collection for the father showed that he had undergone an abdominal ultrasound with evidence of multiple bilateral lesions compatible with angiomyolipomas at the age of 24; in the following years, the angiomyolipomas grew in size so that, at age 52, after a round of embolization, the surgical removal of the largest one (measuring 20 cm in diameter) became necessary (Figure 1). On that occasion, a tissue sample was also retrieved and used for DNA extraction and analysis, together with a fresh blood sample from both father and daughter.

Using the daughter's blood sample as a positive control and targeting the analyses on the known *TSC2* familial variant, both NGS and Sanger sequencing were performed on DNA extracted from the father's peripheral blood and angiomyolipoma samples: low-level mosaicism for this variant was found both in

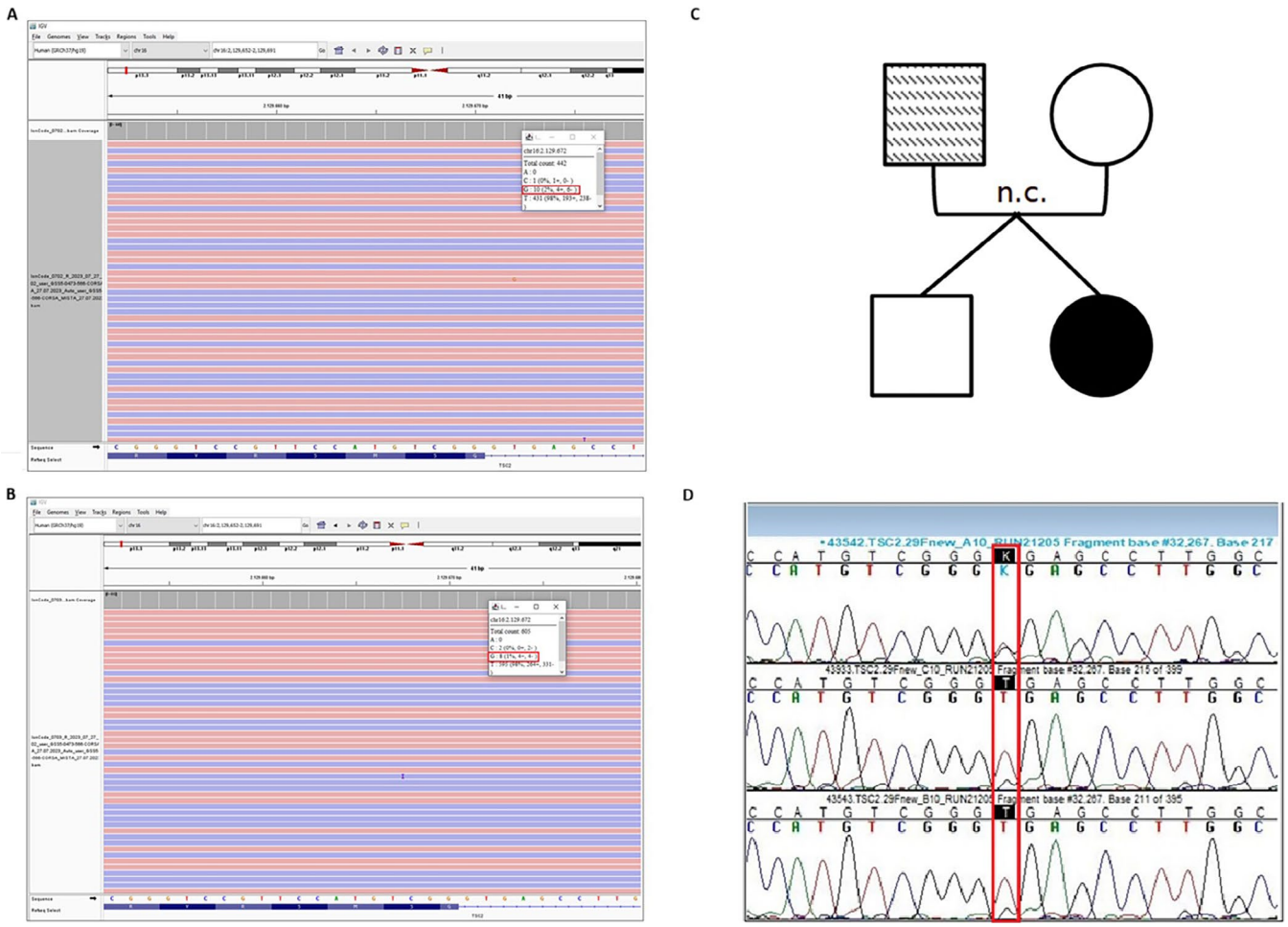

FIGURE 2 | Case 1—Panels A (angiomyolipoma) and B (blood): IGV read counts for *TSC2*: c.3397+2T>G variant. Panel C: family history. Panel D: Sanger validation—top: daughter; middle: father's angiomyolipoma and blood.

the father's angiomyolipoma-derived tissue (VAF 2.26%, 10/442 reads) and blood (VAF 1.65%, 8/605 reads) (Figure 2A,B). The mosaicism was also visible, however slightly, on Sanger sequencing (Figure 2D).

Based on this finding, the father received a formal diagnosis of TSC.

3.1.2 | Case 2

A diagnosis of infantile myofibromatosis (OMIM #228550) was suspected in a male patient with multiple subcutaneous lesions first observed at 1 month of age, histologically defined as myofibromas; skeletal radiological imaging revealed a small (6–7 mm) osteolytic lesion in the proximal metaphyseal–diaphyseal region of the right tibia, which had disappeared at the follow-up examination the following year. At 7 years of age, following the onset of seizures, the patient underwent brain MRI revealing bilateral cortical–subcortical signal abnormalities and subependymal nodules, suggestive of TSC. To confirm this hypothesis, genetic analysis of *TSC1* and *TSC2* genes was conducted using DHPLC and MLPA techniques, failing to identify pathogenic variants. Over the following years, several additional lesions were detected, including a suspected splenic hamartoma, a suspected localization of myofibromatosis in the liver, an osteolytic lesion of the cranial vault histologically defined as a benign myofibroblastic lesion, and an intraneural myofibroma located at the left radial nerve.

The patient came to our attention at 17 years of age and whole exome sequencing (WES) on peripheral blood was performed, detecting the heterozygous de novo PDGFRB(NM_002609.4):c.1681C>T (p.Arg561Cys) pathogenic variant, confirming the diagnosis of infantile myofibromatosis.

On a subsequent dermatological evaluation, multiple angiofibromas on the alae nasi, ectopic angiocheratomas of the hands, and subungual red comets were identified. A biopsy of one of the angiofibromas was performed, and the *TSC1/2* gene sequencing carried out on DNA extracted from this lesion identified the *TSC1*:c.2103_2106dup (p.Leu703ValfsTer4) pathogenic variant with a VAF of 16%, which was not previously identified on peripheral blood DNA. This variant has been previously described in the literature, and it is predicted to cause frameshift with premature termination of the transcript (Tsai et al. 2011).

After the variant was found on DNA extracted from the skin lesion, we reevaluated WES data for this patient; the *TSC1* variant identified in the angiofibroma was only present in very few reads and therefore was not flagged in the original data analysis. To confirm the diagnosis of mosaic TSC, we performed a targeted search for the specific variant in different tissues; in particular, NGS was repeated on peripheral blood and was performed for the first time on saliva and non-lesional skin biopsy: the variant was identified in all three samples, with very low VAFs: 2.5% in non-lesional skin (21/827 reads), 2.2% in blood (23/1025 reads), and 2% in saliva (8/392 reads) (Figure 3).

This established a dual diagnosis of infantile myofibromatosis and mosaic TSC.

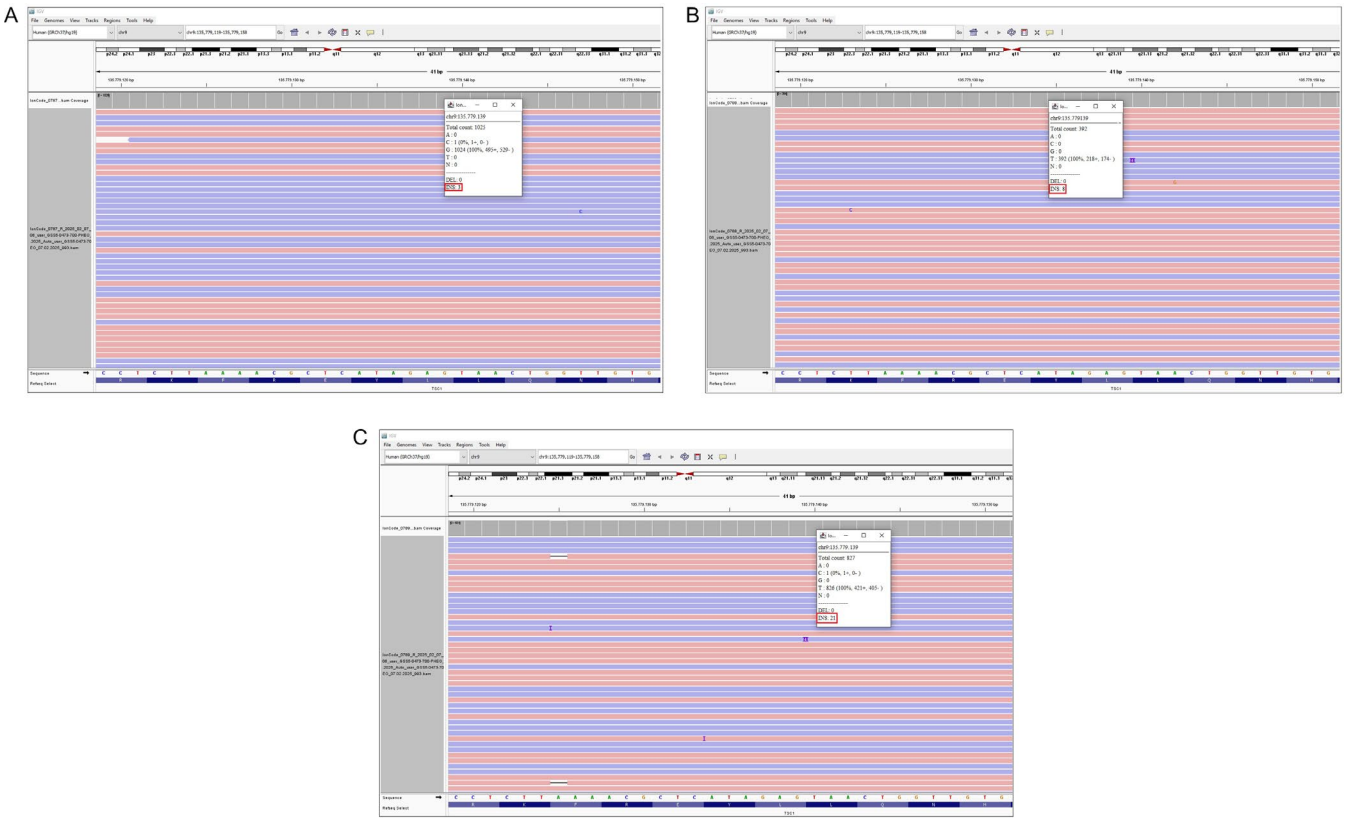

FIGURE 3 | Case 2—Panels A (blood), B (saliva), and C (skin): IGV read counts for *TSC1*: c.2103_2106dup.

TABLE 1 | Literature review including previously published cases of TSC mosaicism—clinical features.

References	No. patients	Reason for suspicion of mosaicism	Skin lesions	Retinal hamartomas	MRI findings	Cardiac rhabdomyoma	LAM	Angiomyolipomas >2	DD/ID	Epilepsy
Chung et al. (2024)	10	CF	8/10	2/10	9/10	2/10	2/10	5/10	2/10	4/10
Klonowska et al. (2023)[a]	95	CF	89/94	15/84	83/93	19/27	10/71	60/94	12/94	37/94
Blasco-Pérez et al. (2023)	8	CF	8/8	2/6	8/8	3/6	0/4	5/8	UK	UK
Treichel et al. (2023)	3	CF	3/3	UK	1/3	UK	3/3	2/3	0/3	0/3
Ye et al. (2022)	16	CF	14/16	0/16	15/16	7/13	1/14	9/16	6/16	10/16
Togi et al. (2022)	17	CF	6/17	0/11	8/12	5/11	2/11	3/14	1/14	3/17
Sasaki et al. (2022)	1	CF	0	UK	1	UK	0	UK	0	0
Manzanilla-Romero et al. (2021)	4	CF	2/4	3/4	4/4	2/4	0/4	1/4	1/4	1/4
Wang et al. (2021)[b]	6	CF (6), AO (2)	1	NA	NA	6/6	NA	0		NA
Uchiyama et al. (2020)	1	AO	0	UK	UK	UK	UK	UK	0	0
Chen et al. (2020)	1	AO	1	UK	0	0	0	0	0	0
Byers et al. (2018)	2	CF	2/2	1/2	2/2	0/2	UK	1/2	1/2	1/2
Verhoef et al. (1999)	6	AO	6/6	UK	4/4	UK	UK	2/6	0/6	0/6

Abbreviations: AO, affected offspring; CF, clinical features but no constitutive pathogenic variant identified; DD, developmental delay; ID, intellectual disability; LAM, lymphangioleiomyomatosis; UK, unknown.
[a]Includes individuals from the following papers: Giannikou et al. (2019), Treichel et al. (2019), Tyburczy et al. (2015).
[b]Includes individuals from the following paper: Chen et al. (2019).

TABLE 2 | Literature review including previously published cases of TSC mosaicism—molecular features.

References	Peripheral blood	Saliva	Buccal swab	Skin lesion	Non-lesional skin	Angiomyolypoma	Other
Chung et al. (2024)	10 (4%)		12 (3%)	8 (HM 5%, AF & SP 11%)			
Klonowska et al. (2023)[a]	92 (2.73%)	63	5	135 (AF 4.27%, UF 9.70%, SP 15.21%)	38	11 (39.6%)	SEGA 1, semen 15, urine 3, normal kidney 1 (11%), normal lymph node 1, fetal tissue 1
Blasco-Pérez et al. (2023)	8 (16%)	1 (38%)	2 (10%)	1 (AF 42%)	0	0	0
Treichel et al. (2023)	3 (2.45%)	1 (1.18%)		3 (AF 10.06%)	0	0	Urine 2 (0.95%)
Ye et al. (2022)	16 (6.8%)	15 (5.5%)	14 (9.9%)	2 (AF 2.5%)	1 (0.06%)		Urine 15 (12.5%), brain 2 (9.8%)
Togi et al. (2022)	17 (6.5%)						
Sasaki et al. (2022)	1 (1%)						SEGA 1
Manzanilla-Romero et al. (2021)	3 (5.3%)		1 (2.6%)	1 (AF 25)	1 (20%)		
Wang et al. (2021)[b]	2 (5.34%)						Cord blood 3 (12.2%), cardiac rhadbomyoma 6 (30.57%), semen 2 (7%)
Uchiyama et al. (2020)	NA						
Chen et al. (2020)	1 (7%)						Semen 1 (20%)
Byers et al. (2018)	2 (~20%)		1 (UK)	2 (HM ~25%, AF ~50%)	2 (~20%)		
Verhoef et al. (1999)	5						

Abbreviations: AF, angiofibroma; HM, hypomelanotic macule; SP, Shagreen patch; UF, ungual fibroma; UK, unknown.
[a]Includes individuals from the following papers: Giannikou et al. (2019), Treichel et al. (2019), Tyburczy et al. (2015).
[b]Includes individuals from the following paper: Chen et al. (2019).

4 | Literature Review

A total of 28 papers were identified as relevant to the scope of this study, 20 of which were included in the final assessment. Regarding clinical features, brain MRI anomalies were found to be the most frequent (87% of cases), followed by cutaneous findings (72% of cases) and renal angiomyolipomas (53% of cases). In the majority of cases, the diagnostic test was NGS (Massive Parallel Sequencing). The most frequently analyzed tissues were skin lesions and peripheral blood, but mosaic variants were also identified in other tissues including saliva, buccal swab, urine, semen, angiomyolipoma, and brain lesions (subependimal giant astrocytomas). Further detail is available in Tables 1 and 2.

5 | Discussion

Mosaicism in TSC individuals is not uncommon, and this has important implications for genetic counseling. Most individuals with TSC mosaicism described in the literature meet the criteria for a clinical diagnosis, but often a diagnosis is only reached after the birth of affected children. Therefore, individuals meeting the clinical criteria for TSC with no pathogenic variants identified by conventional testing should prompt further investigations considering the possibility of mosaicism.

Among mosaic TSC individuals reported in the literature, cutaneous findings are particularly frequent, described in around 70% of individuals and in one of the two cases presented here. Therefore, if an accessible TSC-related lesion is available, a biopsy should be recommended to perform genetic testing with a higher chance of identifying a pathogenic variant.

Notably, tumorigenesis in TSC is believed to follow Knudson's two-hit hypothesis, whereby a second, tissue-specific mutation leads to the inactivation of the wild-type *TSC1/2* allele in individuals who carry a germline (or mosaic) pathogenic variant in the first copy of the gene. Therefore, if a pathogenic variant is only identified in one lesion, it may represent a second genetic hit following a first, cryptic hit. Testing multiple tissues in mosaic individuals enables the differentiation between the first and second genetic hits, particularly in the absence of a family history of affected offspring.

In this work, we described two complex cases of TSC mosaicism. The first proband, despite the low VAFs in peripheral blood and affected tissue, had rapidly growing angiomyolipomas requiring surgery due to a compressive effect and the risk of internal bleeding. The second proband is also affected by myofibromatosis, a condition with clinical features overlapping those of TSC. In both cases, on careful examination, the probands met the clinical criteria for TSC, but standard NGS testing would have been reported as negative were it not for the family history in the first case and for the possibility to analyze multiple tissues in the second. After diagnosis, both individuals were referred for TSC-specific monitoring, and in the second case, genetic counseling was offered to the family to discuss prenatal and/or preimplantation testing.

In conclusion, correctly identifying the first hit and providing a molecular diagnosis to a TSC mosaic individual can have profound repercussions, not only for their management and follow-up, but also for family planning (Ikeda et al. 2021). In patients who meet the clinical diagnostic criteria with no pathogenic variant identified on peripheral blood, particularly when cutaneous lesions are present and accessible, the diagnostic approach may be shifting from the traditional paradigm used in Mendelian disorders toward strategies already employed for other disorders of the mTOR pathway known to be caused by mosaic variants (e.g., *PIK3CA*-related disorder). This more invasive approach, which includes tissue biopsy for molecular analysis, is increasingly justified as targeted therapies become available and because of the implications of a molecular diagnosis for family planning.

Author Contributions

Conceptualization and methodology: I.A., C.P.C., G.I. Data collection and curation: F.Mon., L.C., F.Mod., I.C., V.A., S.L., I.N., E.B., C.R., M.S., D.T. Original draft preparation: I.A., C.P.C. Writing – reviewing and editing: G.I., D.T., C.R., I.N., F.Mod., E.B.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in the Zenodo repository at https://doi.org/10.5281/zenodo.17877780.

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