Genotype-Phenotype Correlation in Tuberous Sclerosis Complex: Insights from Thirty-three Patients from North Macedonia

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Tuberous sclerosis complex (TSC) is a rare autosomal dominant genetic disorder characterized by the development of benign tumors (hamartomas) in multiple organs, including the brain, kidneys, heart, lungs, and skin. These growths can lead to seizures, developmental delay, intellectual disability, cutaneous manifestations, and renal complications.[1] TSC is caused by pathogenic variants in the *TSC1* and *TSC2* genes, which encode the tumor suppressor proteins hamartin and tuberin, respectively. Clinical severity varies widely, ranging from mild manifestations to severe multisystem involvement.[2]

Seventy individuals with suspected TSC were referred for molecular testing, including eight newborns and four fetuses with ultrasound findings suggestive of TSC. Genetic analysis combined multiplex ligation-dependent probe amplification (MLPA; P124, P046, and P337 probe sets) to detect copy number variants (CNVs) and next-generation sequencing (TruSight Cancer panel) to identify single-nucleotide variants (SNVs) in *TSC1* and *TSC2*, following the manufacturers' protocols. When a pathogenic variant was identified, parental confirmation was performed using targeted Sanger sequencing.

A definitive molecular diagnosis was established in 33 patients (47.1%). Two patients had insufficient clinical data for follow-up, and two fetal cases lacked adequate clinical details. The detection rate was lower than the 75–90% reported in other cohorts, likely because this group included patients with subtle or incomplete TSC features.[3] Undetected cases may also involve low-level mosaicism not captured by standard testing, underscoring the need for high-depth sequencing or multi-tissue analysis when clinical suspicion remains high despite negative results.[4,5]

Pathogenic *TSC2* variants predominated (66.7%), consistent with previous reports.[6] The larger size of the gene and its association with more severe phenotypes likely contribute to this difference. Nine patients had inherited mutations, whereas 21 cases were *de novo*. Familial cases were proportionally more common in *TSC1* (45%) than in *TSC2* (21%), supporting the established pattern that *TSC2* variants arise more frequently *de novo*. The overall ~3:1 ratio of *de novo* to inherited cases was consistent with known epidemiological data.[7] Four patients (14, 12, 17, and 19) were identified as mosaic for the detected variant, as indicated by reduced variant allele frequency.

In total, 30 unique pathogenic variants were identified, including 11 novel loss-of-function variants (*TSC1*: c.363+5G>A, c.1142-2A>G, c.1363dupA, c.1498delC, c.1734_1738delinsT, c.1950_1951delTG, c.3113_3119del; and *TSC2*: c.123_127delinsTGAT, c.772A > T, c.2097+2T > G, c.4318delC), all classified as likely pathogenic according to American College of Medical Genetics and Genomics criteria, thereby expanding the known mutational spectrum.[8,9]

Beyond SNVs and small indels, several large multi-exonic *TSC2* deletions were detected (Table 1), including deletions involving exon 1, exons 1–15, and exons 22–41, as well as *TSC2/PKD1* contiguous gene deletions identified in two siblings (Figure 1). These alterations cause tuberin haploinsufficiency and reflect genomic instability at the 16p13.3 *TSC2–PKD1* locus, underscoring the importance of CNV testing (e.g., MLPA or microarray) for detecting large rearrangements that may be missed by sequence-based methods alone.

Data from multiple studies on the genetic landscape of TSC in Southeastern Europe demonstrate a high degree of mutational

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TABLE 1. Clinical Findings and Genetic Variants in Molecularly Diagnosed TSC Cases.

			Major features											Minor features								Other			Result		
	Case	Age	HM	AF or FCP	UF	ShP	MRH	CD	SENs	SEGA	CR	LALM	AM	CSL	DEP	IOF	RAP	MRC	NRH	SBL	SZ	ID	BPP	TSC2:NM_000548.5; TSC1:NM_000368.5	TSC2:NP_000539.2; TSC1:NP_000359.1	Inherit.
TSC1	1	31	-	-	-	-	-	/	/	/	-	-	-	-	-	-	/	-	-	-	+	-	-	c.363+5G > A	/	MM
	2	nb	/	/	/	/	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	c.1142-2A > G	/	DN
	3	8	+	+	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	+	+	+	c.1363dupA	p.Thr455Asnfs*4	DN
	4	9	+	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+	/	/	c.1431_1434del	p.Glu478Lysfs*53	DN
	5	17	+	+	-	-	+	-	+	-	+	-	-	-	-	-	+	-	+	-	+	-	-	c.1498delC	p.Arg500Glufs*32	DN
	6	7	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	+	/	/	+	-	-	c.1498C > T	p.Arg500*	pgm
	7	6/sib fet	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	c.1498C > T	p.Arg500*	pgm
	8	nb	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	+	+	c.1734_1738delinsT	p.Ile580Serfs*48	F
	9	40	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	c.1950_1951del	p.Asp650Glufs*37	DN
	10	9	+	+	+	-	+	-	+	-	+	-	-	-	+	-	-	-	+	-	+	+	+	c.2356C > T	p.Arg786*	DN
	11	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	+	/	/	c.3113_3119del	p.Ser1038Thrfs*51	F
	12	25	+	+	-	-	-	/	/	/	/	/	+	-	-	-	-	/	-	-	+	+	+	c.123_127delinsTGAT	p.Ala42Aspfs*4	DN, mos.30%
TSC2	13	3	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	+	+	-	+	+	+	c.772A > T	p.Lys258*	DN
	14	30	/	+	-	/	/	-	-	-	/	+	+	/	-	/	/	+	/	/	+	+	+	c.1513C > T	p.Arg505*	DN, mos.20%
	15	1	+	+	-	+	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+	+	+	c.1793A > G	p.Tyr598Cys	DN
	16	6 m	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	c.1832G > A	p.Arg611Gln	DN
	17	37	+	+	+	+	/	-	+	+	+	+	+	/	-	-	/	+	+	/	-	-	-	c.1932C > A	p.Cys644*	NA, mos.30%
	18	11	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	c.2097+2T > G	/	M
	19	30	-	+	-	-	-	/	+	-	-	+	+	-	-	-	-	+	/	/	+	-	+	c.3094C > T	p.Arg1032*	DN, mos.20%
	20	3	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	c.3598C > T	p.Arg1200Trp	M
	21	fet	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	c.3693_3696del	p.Ser1232Thrfs*92	NA
	22	6 m	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	-	+	-	+	+	+	c.4318delC	p.Gln1440Serfs*36	DN
	23	32	+	+	+	-	+	+	+	-	-	+	+	-	+	-	-	-	-	+	+	+	+	c.4594C > T	p.Gln1532*	DN
	24	14	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	+	-	+	+	+	c.4925G > A	p.Gly1642Asp	DN
	25	7 m	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	+	-	-	+	+	+	c.4952A > G	p.Asn1651Ser	DN
	26	6 m	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	c.5024C > T	p.Pro1675Leu	DN
	27	9	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	+	+	+	c.5238_5255del	p.His1746_Arg1751del	NA
	28	31	-	+	-	+	/	/	/	/	-	-	/	-	-	-	/	/	/	/	+	+	+	c.5238_5255del	p.His1746_Arg1751del	DN
	29	13	+	+	-	-	/	-	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	del ex1	/	DN
	30	1	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	+	+	+	del ex1-15	/	DN
	31	5	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	+	+	+	del TSC2:ex3,31-42; PKD1:ex.35-46	/	MM
	32	31/ sib	3	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	+	+	+	del TSC2:ex3,31-42; PKD1:ex.35-46	/	MM
	33	2	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+	del ex22-41	/	DN

Novel variants are in bold. HM, hypomelanotic macules; AF, angiofibromas; FCP, fibrous cephalic plaque; UF, ungual fibromas; ShP, shagreen patch; MRH, multiple retinal hamartomas; CD, cortical dysplasia; SENs, subependymal nodules; SEGA, subependymal giant cell astrocytoma; CR, cardiac rhabdomyoma; LALM, lymphangioleiomyomatosis; AM, angiomyolipomas; CSL, confetti skin lesions; DEP, dental enamel pits; IOF, intraoral fibroma; RAP, retinal achromic patch; MRC, multiple renal cysts; NRH, non-renal hamartomas; SBL, sclerotic bone lesions; SZ, seizures; ID, intellectual disability; BPP, behavioral/psychiatric problems; Nb, newborn; fet, fetus; sib, sibling; Inher., inheritance; M, mother; MM, mother mosaic; F, father; pgm, parental gonadal mosaicism; DN, *de novo*.

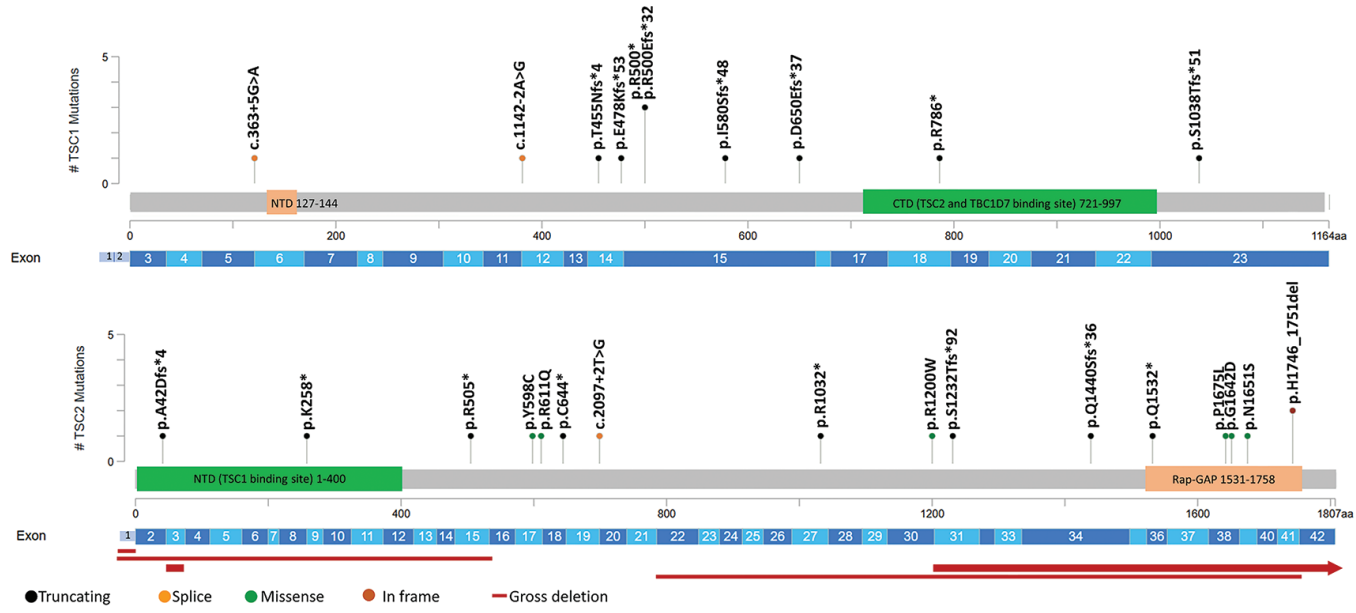

FIG. 1. *TSC1* and *TSC2* variants detected in patients from North Macedonia.

heterogeneity, with most variants being unique to individual patients and many novel variants identified in each cohort; our findings similarly reflect this pattern.[10-13]

A broad spectrum of clinical manifestations was observed in our TSC cohort (Table 1). Despite the defining clinical features of TSC, neurological involvement predominated. The most common symptom was seizures, observed in 90% of *TSC1* patients and 95.2% of *TSC2* patients. This was followed by subependymal nodules, present in 85.7% and 94.7% of *TSC1* and *TSC2* patients, respectively. These findings underscore that both genetic subtypes are associated with significant neurological morbidity, although differences in severity between *TSC1* and *TSC2* cases were noted. Although seizures, intellectual disability, and behavioral problems were more frequent in *TSC2* patients, these differences were not statistically significant ($p > 0.99$, $p > 0.99$, and $p = 0.31$; Fisher's exact test), likely due to the limited sample size. Shagreen patches, angiomyolipomas, lymphangioleiomyomatosis, sclerotic bone lesions, and subependymal giant cell astrocytomas were observed only in patients with *TSC2*, supporting the notion that *TSC2* mutations often result in a more severe and multisystem form of TSC, with broader organ involvement.[2,14]

Two familial cases from our study warrant special mention because of their implications for heredity and genetic counseling in TSC. Cases 31 and 32 involved two siblings who presented with TSC at an early age. Genetic testing revealed an identical large deletion encompassing a portion of the *TSC2* gene and the adjacent *PKD1* gene, along with deletion of exon 3 in *TSC2*. Individuals harboring a *TSC2–PKD1* contiguous gene deletion typically manifest TSC in combination with early-onset autosomal dominant polycystic kidney disease (ADPKD).[15] Indeed, both children exhibited multiple renal cysts consistent with ADPKD. This finding raised the question of how the same deletion occurred twice in a family with initially unaffected parents. Parental testing revealed that the mother carried the same deletion in mosaic form in her blood DNA. Reverse phenotyping identified a small number of renal cysts, suggesting subtle manifestations of ADPKD. In addition, the mother of Case 1 was also mosaic, and her medical history indicated epilepsy between the ages of 13 and 40 years. These examples illustrate somatic mosaicism in TSC, which is well documented in the literature. Our findings underscore that when two children in a family are affected by TSC without an apparent family history, parental testing for mosaic mutations should be considered. This includes the use of sensitive detection methods or analysis of multiple tissues, as such findings have important implications for recurrence risk assessment and clinical surveillance.

The third family (Cases 6 and 7) highlights the issue of gonadal mosaicism. A girl with a *TSC1* non-sense mutation initially appeared to carry a *de novo* variant (*TSC1*: c.1498C > T); however, the same mutation was subsequently detected prenatally in a sibling, indicating parental germline mosaicism. This observation reinforces an important genetic counseling principle: even when a TSC mutation appears *de novo*, there remains a low but clinically significant (~1–3%) recurrence risk in future pregnancies due to possible parental gonadal mosaicism.[2,16] Therefore, offering prenatal or preimplantation genetic testing to families with an apparently sporadic TSC case is advisable, as it enables the detection of otherwise unexpected recurrence. These cases emphasize the importance of comprehensive family evaluation—including clinical assessment and targeted genetic testing—to uncover subclinical TSC in parents, as recommended by current TSC Consensus guidelines.[14]

Although our study focused on the genetic and clinical characteristics of TSC, translating these findings into patient management is equally

important. As a complex and lifelong condition, TSC requires a multidisciplinary approach. In recent years, treatment has advanced significantly with the introduction of targeted therapies addressing the underlying molecular mechanism of TSC, particularly inhibition of the hyperactivated mammalian target of rapamycin (mTOR) pathway.[17] In addition, Rheb inhibitors are being investigated as regulators of mammalian target of rapamycin complex 1 activity.[18] Because epilepsy is highly prevalent and often begins in infancy, conventional antiseizure medications remain essential. Cannabidiol has also emerged as an approved adjunctive therapy for refractory TSC-associated epilepsy in several countries.[19] Although no single therapy is curative, a combination of antiepileptic drugs, mTOR-targeted therapies, dietary and surgical interventions, and emerging agents can substantially reduce seizure burden. In our cohort, most patients with epilepsy received combination therapy with mTOR inhibitors and antiepileptic medications.

Equally important is the management of TSC-associated neuropsychiatric disorders, including intellectual disability, autism spectrum disorder, attention-deficit/hyperactivity disorder and other behavioral conditions. These require structured support through behavioral therapy, educational interventions, and, when appropriate, psychiatric management under specialist care.

Current TSC guidelines emphasize proactive surveillance and preventive care. Routine brain magnetic resonance imaging, periodic renal imaging, and regular dermatologic, ophthalmologic, and pulmonary evaluations enable early detection and timely management of complications. Together with modern therapeutic approaches, such surveillance strategies allow many TSC-related complications to be prevented or mitigated.

In conclusion, our findings contribute to the growing understanding of genotype–phenotype correlations in TSC and highlight the importance of integrating genetic diagnosis with vigilant, up-to-date clinical management to optimize patient outcomes.

Ethics Committee Approval: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Subcommittee of the Macedonian Academy of Science and Arts for Medicine, Pharmacy, Veterinary Medicine and Dentistry (approval number: 03-400/3, date: 09.12.2025).

Informed Consent: Written informed consent was obtained from each patient.

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