Neurology Asia 2026; 31(2) : 771 – 779

Clinical and molecular spectrum of *TSC1* and *TSC2* mutations in pediatric tuberous sclerosis complex: Report of five novel variants

[1]Sevgi Yimenicioglu, [2]Ayca Kocaaga, [1]Cefa Nil Arslan Karademir, [3]Yasar Bildirici

[1]Department of Pediatric Neurology, Health Sciences University Eskisehir City Hospital, Eskişehir, Turkey; [2]Department of Medical Genetics, Health Sciences University Eskisehir City Hospital, Eskişehir, Turkey; [3]Department of Pediatrics, Health Sciences University Eskisehir City Hospital, Eskişehir, Turkey

Abstract

Background: Tuberous sclerosis complex (TSC) affects multiple systems. We evaluate the patients with TSC clinically in detail and, if possible, make genotype–phenotype correlations in patients with pathogenic variants in the *TSC1* and *TSC2* genes. *Methods:* In this retrospective study, pathogenic germline variants in the *TSC1* and *TSC2* genes were identified using a combination of direct sequencing and multiplex ligation-dependent probe amplification (MLPA). There were 15 males (53.6%) and 13 females (46.4%). *Results:* Twelve patients (42.8%) carried genetic mutations. All detected variants were classified as pathogenic or likely pathogenic, and five variants (41.6%)—four in the *TSC1* gene and one in the *TSC2* gene—had not been previously reported. Patients were divided into two groups according to the current diagnostic criteria: definite TSC (17 patients) and possible TSC (11 patients). The frequency of cortical tubers, renal cysts, adenoma sebaceum, and definite diagnosis increased with age ($p = 0.023$, 0.002, 0.02, and 0.013, respectively). Cortical tubers, renal cysts, and angiomyolipomas were observed more frequently in patients with genetic mutations ($p = 0.001$, 0.002, and <0.001, respectively). Epilepsy was common in patients with intellectual disability ($p = 0.03$).
Conclusion: Patients with genetic mutations frequently exhibit cortical tubers, renal cysts, and angiomyolipomas. Radiological examinations should be performed repeatedly over time, as cortical tubers, adenoma sebaceum, renal cysts, and definite diagnoses tend to increase with age. In addition, patients with epilepsy should be evaluated for intellectual disability.

Keywords: Multiplex ligation-dependent probe amplification, tuberous sclerosis complex, *TSC1* gene, *TSC2* gene, pathogenic.

INTRODUCTION

Tuberous sclerosis complex (TSC; OMIM #191100) is an autosomal dominantly inherited multisystemic disease caused by mutations in the *TSC1* and *TSC2* genes.[1] The *TSC1* gene consists of 23 exons and produces an 8.5 kilobase (kb) transcript, from which a 130 kDa protein product, called hamartin, is derived. The *TSC2* gene contains 42 exons distributed over a genomic area of approximately 40 kb, produces a 5.5 kb transcript, and encodes a 200 kDa protein called tuberin.[2] In cells harboring mutant hamartin or tuberin, mTOR signaling is dysregulated, leading to abnormal cell development and differentiation and the formation of various lesions characteristic of TSC.[3] Approximately 80% of individuals initially diagnosed with definite TSC harbor pathogenic variants in the *TSC1* or *TSC2* genes, while some of the remaining patients exhibit mosaicism or carry deep intronic mutations.[4,5] For clinical diagnosis, TSC manifestations are categorized into two subgroups: major and minor features. Major features include angiofibromas, fibrous cephalic plaques, hypomelanotic macules, ungual fibromas, cortical dysplasia, shagreen patches, multiple retinal hamartomas, subependymal nodules or giant cell astrocytomas, cardiac rhabdomyomas, and angiomyolipomas.

Address correspondence to: Dr. Sevgi Yimenicioglu, Department of Pediatric Neurology, Health Sciences University Eskisehir City Hospital, Eskişehir Şehir Hastanesi, 71 Evler Mahallesi, Çavdarlar Sokak, TR 26080 Odunpazarı/Eskişehir, Türkiye. e-mail: sevgifahri@yahoo.com

Date of Submission: 26 September 2025; Date of Acceptance: 20 February 2026

https://doi.org/10.54029/2026jwy

Minor features include confetti-like skin lesions, dental pits, intraoral fibromas, sclerotic bone lesions, and multiple renal cysts. The presence of two major features, or one major feature plus two or more minor features, is sufficient for a definite clinical diagnosis.[6] The detection of a pathogenic germline variant in the *TSC1* or *TSC2* genes confirms a definite diagnosis of TSC.[7]

The aim of this study was to provide a detailed clinical characterization of children with TSC and, where possible, to evaluate genotype–phenotype correlations in patients harboring pathogenic variants in the *TSC1* and *TSC2* genes.

METHODS

Patient ascertainment and assessment

Twenty-eight unrelated children, referred to the Medical Genetics Department with a definite or possible diagnosis of TSC between 2020 and 2025, were included in the study. Informed consent was obtained from the parents or legal guardians of the patients. Medical records for each patient were reviewed to collect data on birth and family history, seizure types (if present), neurodevelopment, central nervous system manifestations, cardiac anomalies, renal involvement, and skin manifestations.

Next generation sequencing

Genomic DNA was isolated from whole blood according to the manufacturer's instructions (Qiagen, Hilden, Germany). Full-length sequencing analysis of the *TSC1* and *TSC2* genes, including targeted exons and intronic regions, was performed using next-generation sequencing (MiSeq, Illumina). All coding regions and exon–intron boundaries of *TSC1* (NM_000368.5) and *TSC2* (NM_000548.5) were amplified by PCR using specific primers. The Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA) was used for target enrichment in accordance with the manufacturer's instructions. The hg19 human reference genome (Genome Reference Consortium GRCh37) served as the reference sequence. Sequencing data were analyzed using the Integrative Genomics Viewer (IGV), and coverage analysis was performed with the DRAGEN coverage analysis plugin. A minimum coverage of 20× for targeted bases was considered reliable for variant calling. The tools MutationTaster, SIFT, PolyPhen-2, DANN, and REVEL were utilized to predict the pathogenicity of the identified variants. The variants were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines, taking into account population frequencies from the gnomAD, ClinVar, and dbSNP databases. All variants were confirmed in patients by Sanger sequencing. In patients with identified variants, Sanger sequencing was also performed on the parents for segregation analysis, except for P17 and P25.

Statistical analysis

Descriptive statistics were presented using frequency, percentage, mean, standard deviation, median(minimum – maximum). The assumption of normality was checked using the Shapiro-Wilk test by examining histograms, q-q plots, skewness, and kurtosis. The Mann-Whitney U test was used to analyze the differences between numerical data in the two groups. Relationships between categorical data were analyzed using Pearson's chi-square test when the proportion of cells with an expected value of less than 5 was less than 20% and Fisher's exact test when it was greater than 20%. P values less than .05 were considered statistically significant.

RESULTS

This study enrolled twenty-eight children with TSC from unrelated families: 15 males (53.6%) and 13 females (46.4%). The mean age was 8.54 ± 6.08 years. The median age was 8.5 (1 -18). A positive family history was detected in four patients (14.2%). The majority of patients (24; 85.8%) presented with hypomelanotic macules. Epilepsy was observed in 21 patients (75%). Developmental delay or mild intellectual disability was noted in 10 patients (35.7%), and atypical autism in one patient (3.5%). Renal angiomyolipomas were detected in eight patients (28.6%), and renal cysts in six patients (21.4%). Brain MRI findings were normal in 21 patients, while cortical tubers were observed in seven patients and subependymal nodules in one patient. Cardiac abnormalities were identified in 11 patients (39.2%), including cardiac rhabdomyomas in two patients (7%). The clinical features of the study group, according to pathogenic variants, are summarized in Table 1and Table 2.

The overall *TSC1*/*TSC2* gene variant detection rate was 42.8% (12/28). Of these, 7 patients (58.3%) carried *TSC1* gene (NM_000368.5) mutations and 5 (41.7%) carried *TSC2* gene

(NM_000548.5) mutations. All detected variants were pathogenic or likely pathogenic, and 5 (41.6%) were novel. The novel variants were *TSC1* c.786dup (p.Ser263LeufsTer10), c.891dup (p.Ala298CysfsTer2), c.452T>C (p.Leu151Pro), c.668T>C (p.Met223Thr), and *TSC2*c.2056T>C (p.Tyr686His) (Table 3). No recurrent mutations were detected.

TSC1 gene variants included one nonsense and three frameshift mutations, all resulting in protein truncation. There were also two missense and one intronic variant in the *TSC1* gene. *TSC2* gene variants comprised two missense, two nonsense, and one frameshift mutation. Four were inherited mutations (three paternal and one maternal), while five occurred de novo (Table 2). Of the 16 patients with negative sequencing results, 5 underwent *TSC1*/*TSC2* MLPA testing, but no large deletions or duplications were detected. Eleven patients were either not contacted again or did not return for MLPA testing. Based on diagnostic criteria, patients were classified as possible or definite TSC (Table 2). Mutations were identified in 9 of 14 definite TSC patients (five in *TSC1* and four in *TSC2*) and in 3 of 14 possible TSC patients (two in *TSC1* and one in *TSC2*). Patients 7, 19, and 26 initially had possible TSC. They were later included in the definite TSC group with identified mutations. Two of these three patients, patient 7 and patient 26, had novel mutations. Segregation analysis could be performed in 10 of the 12 patients in whom mutations were detected. Of these variants, 6 were inherited de novo, three were paternal, and one was maternal (Table 2).

There were 12 patients with genetic mutations and 16 patients without. The median age of the 17 patients with a definite diagnosis was significantly higher than that of possible TSC ($U = 41$, $z = -2.48$, $p = 0.013$). The median age of the 12 patients with genetic mutations was significantly lower than that of the 16 patients without genetic mutations ($U = 48.5$, $z = -2.26$, $p = 0.024$).

The median age of the five patients with adenoma sebaceum was significantly higher than that of the 23 patients without adenoma sebaceum. Adenoma sebaceum was more commonly seen as the age increased ($U = 19$, $z = -2.32$, $p = 0.02$). Cortical tubers were identified in seven patients (7/28; 25%), with two harboring *TSC1* mutations and five carrying *TSC2* mutations. The median age of the 7 patients with cortical tubers was significantly higher than that of the 21 patients without cortical tubers ($U = 31$, $z = -2.26$, $p = 0.023$). There were six patients with renal cysts and 22 patients without renal cysts. The median age of the six patients with renal cysts was significantly higher than that of the patients without renal cysts ($U = 11.5$, $z = -3.06$, $p = 0.002$).

In the study, there were six females without a genetic mutation and seven females with a genetic mutation. There were 10 males without a genetic mutation and five males with a genetic mutation. No significant difference was found between genetic mutation and gender ($p=0.27$). Five of those with a family history (100%) had a genetic mutation. Seven of the 23 individuals without a family history had a genetic mutation, representing 30.4%. Genetic mutations were more common in those with a family history ($p = 0.08$). When examining the family history and those with and without a definite diagnosis, all five patients with a family history of tuberous sclerosis had a definite diagnosis. Of the 23 patients without a family history, 12 (52.2%) had a definite diagnosis, and 11 had a probable diagnosis. There was no significant difference between family history and definite diagnosis ($p=0.12$).

Twelve (50%) of the 24 patients with hypomelanotic macules had genetic mutations, while all of the 4 patients without hypomelanotic macules had genetic mutation rates. No significant difference was found between genetic mutations and the presence of hypomelanotic macules ($p = 0.11$). Four of the five patients (80%) with adenoma sebaceum had a genetic mutation. Eight of the 23 patients (34.8%) without adenoma sebaceum had a genetic mutation. No significant difference was found between the genetic mutation and adenoma sebaceum ($p = 0.13$). Three (75%) of the four patients with facial angiofibroma had a genetic mutation. Nine (37.5%) of the 24 patients without facial angiofibroma had a genetic mutation. There was no significant difference between the patients with genetic mutations and those with facial angiofibroma ($p = 0.28$).

Two (66.7%) of the three patients with shagreen patches had a genetic mutation. Ten (40%) of the 25 patients without shagreen patches had a genetic mutation. There was no significant difference between the shagreen patch and having the genetic mutation ($p = 0.56$).

All of the seven patients with cortical tubers had genetic mutations. Of the 21 patients without cortical tubers, five (23.8%) had genetic mutations. A significant difference was found

Table 1: Clinical features of the patients according to pathogenic variants in *TSC1*/*TSC2*

Clinical Features		Pathogenic *TSC1* or *TSC2* variant (n)	No pathogenic *TSC1* or *TSC2* variant (n)	*P-value*
Gender	Male	5	10	0.27
	Female	7	6	
Family History	Present	5	-	0.08
	Absent	7	16	
Hypomelanotic macules	Present	12	12	0.11
	Absent	-	4	
Adenoma sebaceum	Present	4	1	0.13
	Absent	8	15	
Facial angiofibroma	Present	3	1	0.28
	Absent	9	15	
Shagreen patches	Present	2	1	0.56
	Absent	10	15	
Cortical tubers	Present	7	-	0.001
	Absent	5	16	
Subependymal nodules	Present	1	-	0.42
	Absent	11	16	
Mental Retardation	Present	5	5	0.69
	Absent	7	11	
Epilepsy	Present	10	11	0.66
	Absent	2	7	
Renal Cysts	Present	6	-	0.0027
	Absent	6	16	
Angıomyolıpoma	Present	8	-	<0.001
	Absent	4	16	
Rhabdomyoma	Present	2	-	0.17
	Absent	-	26	

between the presence of cortical tubers and the occurrence of genetic mutations. Cortical tubers were seen mostly with the patients with genetic mutations (p = 0.001).

When examining the relationship between mental retardation and genetic mutations, five of 10 patients (50%) with mental retardation had genetic mutations, while seven of 18 patients (38.9%) without mental retardation had genetic mutations. There was no significant difference between mental retardation and having genetic mutations (p = 0.69).

Ten of 21 patients (47.6%) with epilepsy had genetic mutations, while two of 7 patients (28.6%) without epilepsy had genetic mutations. There was no significant difference between epilepsy and genetic mutations (p = 0.66).

When examining genetic mutations in patients with subependymal nodules, one patient with subependymal nodules harbored a genetic mutation, whereas 11 of 27 patients (40.7%) without subependymal nodules carried mutations. No significant difference was observed between the presence of subependymal nodules and genetic mutations (p = 0.42). Six patients had renal cysts, all of whom carried genetic mutations, while six of 22 patients (27.3%) without renal cysts had mutations. A significant difference was found between renal cysts and genetic mutations (p = 0.002). Eight patients had angiomyolipomas, and all eight carried genetic mutations. Among the 20 patients without angiomyolipomas, four (20%) had mutations. There was a significant difference between angiomyolipomas and

Table 2: Genotypic and phenotypic features of patients with germline mutation of *TSC1*/2 genes

Patient ID	Sex	Age	Gene mutation/location	Origin of the mutation	Mutation type	Publication	In-Silico Predictors	Diagnostic status*
P1	M	8	(-)	(-)	(-)	(-)	(-)	Possible
P2	M	12	TSC1(NM_000368.5): chr9-135781528 c.1439-2A>C, p.? intron 14	de novo	Intronic	reported	Likely Pathogenic	Definite
P3	F	18	TSC1(NM_000368.5): chr9-135787795 c.786dup; p.(Ser263Leufs*10) exon 9	de novo	Frameshift	Novel, Clinvar: (-) LOVD: (-)	Likely Pathogenic	Definite
P4	F	1	(-)	(-)	(-)	(-)	(-)	Definite
P5	M	8	(-)	(-)	(-)	(-)	(-)	Definite
P6	F	13	TSC1(NM_000368.5): chr9-135787690 C>CA c.891dup; p.(Leu298AlafsTer39) exon 9	paternal	Frameshift	Novel, Clinvar:(-) LOVD: (-)	Likely Pathogenic	Definite
P7	M	3	TSC1(NM_000368.5): chr9-135798791 A>G c.452T>C;p.(Leu151Pro) exon 6	de novo	Missense	Novel, Clinvar: (-) LOVD: (-)	Likely Pathogenic	Definite
P8	F	11	(-)	(-)	(-)	(-)	(-)	Possible
P9	F	11	(-)	(-)	(-)	(-)	(-)	Possible
P10	F	9	(-)	(-)	(-)	(-)	(-)	Possible
P11	M	2	(-)	(-)	(-)	(-)	(-)	Possible
P12	M	1	(-)	(-)	(-)	(-)	(-)	Possible
P13	M	8	TSC2(NM_000548.5): chr16-2131680 C>CT c.3696dupT; p.(Asn1233*) (rs137854210) exon 31	paternal	Frameshift	reported	Pathogenic	Definite

Table 2: (continued)

Patient ID	Sex	Age	Gene mutation/location	Origin of the mutation	Mutation type	Publication	In-Silico Predictors	Diagnostic status*
P14	F	2	(-)	(-)	(-)	(-)	(-)	Possible
P15	F	18	TSC1(NM_000368.5): c.668T>C; p.(Met223Thr) exon 8	paternal	Missense	Novel, Clinvar: (-) LOVD: (-)	Likely Pathogenic/VUS	Definite
P16	M	9	(-)	(-)	(-)	(-)	(-)	Definite
P17	M	17	TSC2(NM_000548.5): c.5413G>T; p.(Glu1805Ter) (rs376017665) exon 42	NA	Nonsense	reported	Pathogenic	Definite
P18	M	4	(-)	(-)	(-)	(-)	(-)	Possible
P19	F	1	TSC1(NM_000368.5): c.1960C>T; p.(Gln654Ter) (rs75820036) exon 15	de novo	Nonsense	reported	Pathogenic	Definite
P20	F	18	TSC1(NM_000368.5): c.1525C>T; p.(Arg509*) (rs118203542) exon 15	de novo	Frameshift	reported	Pathogenic	Definite
P21	M	2	(-)	(-)	(-)	(-)	(-)	Possible
P22	M	14	(-)	(-)	(-)	(-)	(-)	Definite
P23	M	2	(-)	(-)	(-)	(-)	(-)	Possible
P24	M	1	(-)	(-)	(-)	(-)	(-)	Possible
P25	F	9	TSC2(NM_000548.5): c.5353A>C; p.(Thr1785Pro) (rs1555441455) exon 42	NA	Missense	reported	Likely Pathogenic	Definite

Table 2: (continued)

Patient ID	Sex	Age	Gene mutation/location	Origin of the mutation	Mutation type	Publication	In-Silico Predictors	Diagnostic status*
P26	F	17	TSC2(NM_000548.5): c.2056T>C; p.(Tyr686His) exon 19	de novo	Missense	Novel, Clinvar:(-) LOVD: (-)	Likely Pathogenic/VUS	Definite
P27	F	15	(-)	(-)	(-)	(-)	(-)	Definite
P28	M	5	TSC2(NM_000548.5): c.2098G>T; p.(Glu700Ter) (rs45496402) exon 20	maternal	Nonsense	reported	Pathogenic	Definite

*Diagnostic status was grouped as possible and definite due to diagnostic criteria. LOVD: Leiden Open Variation Database; NA: not available; VUS: variant of uncertain significance.

having genetic mutations ($p < 0.001$). Regarding rhabdomyomas, two patients with rhabdomyomas had genetic mutations, whereas 26 patients without rhabdomyomas carried mutations. No significant difference was observed between rhabdomyoma presence and genetic mutations ($p = 0.17$) (Table 1). Concerning hypomelanotic macules and definite diagnoses, 24 patients had hypomelanotic macules; among them, 15 (62.5%) had a definite diagnosis, while nine did not. Four patients did not have hypomelanotic macules, two of whom had a definite diagnosis. No significant difference was found between hypomelanotic macules and definite diagnosis ($p = 1.00$).

Regarding adenoma sebaceum, 4 of 5 patients (80%) with the lesion had a definite diagnosis, whereas 13 of 23 patients (56.5%) without adenoma sebaceum had a definite diagnosis. No significant difference was observed between adenoma sebaceum and definite diagnosis ($p = 0.61$). Among patients with facial angiofibroma, 3 of 4 (75%) had a definite diagnosis, compared with 14 of 24 patients (58.3%) without facial angiofibroma. No significant difference was found between facial angiofibroma and definite diagnosis ($p = 1.00$). Three patients had shagreen patches, two of whom (66.7%) had a definite diagnosis. Among 25 patients without shagreen patches, 15 (60%) had a definite diagnosis. No significant relationship was observed between the presence of shagreen patches and definite diagnosis ($p = 1.00$).

All seven patients with cortical tubers had a clinically definite diagnosis, compared with 10 of 21 patients (47.6%) without cortical tubers. A significant difference was observed between the presence of cortical tubers and a definite diagnosis ($p = 0.023$), indicating that cortical tubers support a definite diagnosis. Among patients with angiomyolipomas, all eight had a definite diagnosis, whereas nine of 20 patients (45%) without angiomyolipomas had a definite diagnosis. The presence of angiomyolipomas significantly supported a diagnosis of tuberous sclerosis ($p = 0.01$).

All 10 patients with intellectual disability had epilepsy, whereas 11 of 18 patients (61.1%) without intellectual disability had epilepsy. A significant difference was observed between intellectual disability and epilepsy ($p = 0.03$). Echocardiographic findings were abnormal in 11 patients (39.2%) and normal in 17 patients (60.8%). Cardiac abnormalities included rhabdomyoma in two patients (7.0%), mitral

valve prolapse in two patients (7.0%), mitral regurgitation in one patient (3.5%), patent foramen ovale in five patients (17.8%), and atrial septal defect in one patient (3.5%).

DISCUSSION

In the present study, the most frequent clinical features were hypomelanotic macules, seizures, renal abnormalities, and neurodevelopmental delay. Pathogenic variants in *TSC1* or *TSC2* genes were identified in 42.8% (12/28) of patients. Although we used combined molecular methodology, there were five cases that met the criteria for a definite TSC diagnosis but in which no mutations were detected. Hung et al. achieved a 76% diagnostic rate in adult patients with TSC using a combination of direct sequencing and MLPA testing.[3] Previous studies have indicated that diagnosis rates can be as high as 86%.[2,8] The lower detection rate in our cohort may reflect the limited use of MLPA in sequencing-negative cases. *TSC1* gene mutations (58.3%) were significantly more common than *TSC2* gene mutations.[4] Existing studies on TSC patients indicate a greater prevalence of *TSC2* mutations relative to *TSC1* alterations.[5,6,9] However, there are also studies showing the opposite. The share of *TSC1* mutations was found to be higher among TSC patients in the Japanese population.[10] Frameshift and nonsense variants represent the most common mutational spectrum reported in the literature.[5,7] In our study, among the 12 detected variants, five (41.6%) were novel and had not been described in the literature before.

In our study, the positive family history was 14.2% and is consistent with other reports (11-38%).[11] Establishing genotype–phenotype correlations is difficult due to the age-dependent manifestation of TSC-related symptoms. We found that demonstrating cortical tubers, renal cysts, adenoma sebaceum, and definite diagnosis significantly increased with increasing age. We found that cortical tubers were seen more frequently with patients having genetic mutations than those without genetic mutations. The occurrence of cortical and subcortical tubers has been reported with variable frequency in the literature.[12,13]

Our data are consistent with these findings, and no cognitive impairment was detected in *TSC1* patients, while 80% (4/5) of *TSC2* patients had mild learning difficulties. Furthermore, variants in *TSC2* have been associated with an increased risk of intellectual disability.[14,15] One (3.5%) of these patients had atypical autism. Previous studies have reported a wide range of autism prevalence in TSC (6–69%).[14,16] The frequency of epilepsy in our cohort was 75% (21/28), consistent with previous reports indicating that 62–93% of patients with TSC experience epilepsy.[1] We found that epilepsy was higher in the patients who had mental retardation than those who did not have it. We observed that renal angiomyolipoma and renal cysts were more frequent in patients harboring a pathogenic variant in *TSC1* or *TSC2* (9/16; 56.25%), which is consistent with findings from previous studies.[12,13] Cardiac rhabdomyoma, a clinical feature whose frequency increases with age in TSC patients, was observed in only two individuals in our pediatric cohort. One patient harbored a *TSC1* mutation, whereas the other carried a *TSC2* mutation. Previous studies have reported that cardiac rhabdomyomas are more frequently observed in individuals with *TSC2* mutations.[9,17] Our data were insufficient to confirm this association.

The differential diagnosis of tuberous sclerosis includes neurofibromatosis type 1 (NF Type 1), Sturge-Weber syndrome, hypomelanosis of Ito, Von Hippel-Lindau disease, and multiple endocrine neoplasia.[18] In our study, there were no patients with sufficient café au lait spots to support the diagnosis of NF Type 1 and meet the criteria. However, there were no patients with port wine spots, which are more prominent on the face, as in Sturge-Weber syndrome. The patients did not have hypopigmented spots consistent with the dermatomal spread suggestive of hypomelanosis of Ito. There were no kidney or brain tumors with clinical signs of von Hippel-Lindau disease. However, no patients had endocrine tumors suggestive of multiple endocrine neoplasia.[18]

This study has limitations, including its small sample size, single-center retrospective design, and the lack of MLPA testing in some patients whose initial sequencing results were negative. However, MLPA detects large deletions or duplications in approximately 3% of TSC patients, and therefore contributes only marginally to the overall diagnostic yield [7]. In our study, MLPA was applied selectively in cases where sequencing did not identify pathogenic variants. Due to logistical and financial constraints, MLPA could not be performed in all eligible patients. Despite this limitation, the overall genetic diagnostic yield remained consistent with previous studies. Furthermore,

the higher variant detection rate among patients with definite TSC (64.2%) compared to possible TSC (21.4%) underscores the strength of clinical diagnostic criteria in guiding molecular testing.

In conclusion, we identified five novel variants in a pediatric cohort of patients with both definite and possible TSC, including four in *TSC1* and one in *TSC2*. Our findings highlight the importance of applying both direct sequencing and MLPA testing for comprehensive genetic analysis.

DISCLOSURE

Ethics: The study adhered to the Declaration of Helsinki and received approval from the Local Ethics Committee (ESH/BAEK/2025/181).

Financial support: None

Conflict of interest: None

REFERENCES

1. Man A, Di Scipio M, Grewal S, *et al.* The genetics of tuberous sclerosis complex and related mTORopathies: Current understanding and future directions. *Genes (Basel)* 2024; 15(3):332. doi: 10.3390/genes15030332
2. Meng Y, Yu C, Chen M, *et al.* Mutation landscape of *TSC1*/*TSC2*in Chinese patients with tuberous sclerosis complex. *J Hum Genet* 2021 ;66(3):227-36. doi: 10.1038/s10038-020-00839-0
3. Hung CC, Su YN, Chien SC, *et al.* Molecular and clinical analyses of 84 patients with tuberous sclerosis complex. *BMC Med Genet* 2006;7:72. doi: 10.1186/1471-2350-7-72
4. Chen CS, Aylett CHS. New insights into tuberous sclerosis complex: from structure to pathogenesis. *Front Cell Dev Biol* 2025 ;13:1595867. doi: 10.3389/fcell.2025.1595867
5. Feliciano DM. The neurodevelopmental pathogenesis of tuberous sclerosis complex (TSC). *Front Neuroanat* 2020;14:39. doi: 10.3389/fnana.2020.00039. eCollection 2020
6. Northrup H, Krueger DA. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol* 2013;49(4):243-54. doi: 10.1016/j.pediatrneurol.2013.08.001
7. Reyna-Fabián ME, Hernández-Martínez NL, Alcántara-Ortigoza MA, *et al*. First comprehensive *TSC1*/*TSC2*mutational analysis in Mexican patients with tuberous sclerosis complex reveals numerous novel pathogenic variants. *Sci Rep* 2020;10(1):6589. doi: 10.1038/s41598-020-62759-5.
8. Boronat S, Caruso P, Thiele EA. Absence of subependymal nodules in patients with tubers suggests possible neuroectodermal mosaicism in tuberous sclerosis complex. *Dev Med Child Neurol* 2014;56(12):1207-11. doi: 10.1111/dmcn.12523.
9. Pal D, Forster N, Madan M, Whitney R, Farncombe KM, Kim RH. Retrospective analysis of Canadian adults with tuberous sclerosis complex. *Can J Neurol Sci* 2024;51(5):636-43. doi: 10.1017/cjn.2023.332
10. Wang S, Sun H, Wang J, *et al*. Detection of *TSC1*/*TSC2*mosaic variants in patients with cardiac rhabdomyoma and tuberous sclerosis complex by hybrid-capture next-generation sequencing. *Mol Genet Genomic Med* 2021;9(10):e1802. doi: 10.1002/mgg3.1802
11. Chopra M, Lawson JA, Wilson M, *et al.* An Australian tuberous sclerosis cohort: are surveillance guidelines being met? *J Paediatr Child Health* 2011;47(10):711-6. doi: 10.1111/j.1440-1754.2011.02038.x.
12. Kovesdi E, Ripszam R, Postyeni E, *et al*. Whole exome sequencing in a series of patients with a clinical diagnosis of tuberous sclerosis not confirmed by targeted *TSC1*/*TSC2* sequencing. *Genes (Basel)* 2021;12(9):1401. doi: 10.3390/genes12091401.
13. Ng SY, Luk HM, Hau EW, *et al*. Genotype/phenotype correlation in 123 Chinese patients with tuberous sclerosis complex. *Eur J Med Genet* 2022;65(10):104573. doi: 10.1016/j.ejmg.2022.104573.
14. Alsowat D, Whitney R, Hewson S, *et al*. The phenotypic spectrum of tuberous sclerosis complex: A Canadian cohort. *Child Neurol Open* 2021;8:2329048x211012817. doi: 10.1177/2329048X211012817
15. Specchio N, Pietrafusa N, Trivisano M, *et al*. Autism and epilepsy in patients with tuberous sclerosis complex. *Front Neurol* 2020;11:639. doi: 10.3389/fneur.2020.00639.
16. Ramani R, Fatima B, Hussain A, *et al.* The clinical interface of tuberous sclerosis complex and autism spectrum disorder: insights and future directions. *Neurol Sci* 2025;46(6):2571-80. doi: 10.1007/s10072-025-08065-2.
17. Gąssowska-Dobrowolska M, Czapski GA, Cieślik M, *et al*. Microtubule cytoskeletal network alterations in a transgenic model of tuberous sclerosis complex: Relevance to autism spectrum disorders. *Int J Mol Sci* 2023;24(8):7303. doi: 10.3390/ijms24087303.
18. Rout P, Thomas A. Tuberous sclerosis. [Updated 2025 Jun 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK538492/?utm_source