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Case Series

Tuberous sclerosis complex type 2 in a subgroup of 7 pediatric patients

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ABSTRACT

Tuberous sclerosis complex (TSC) is a multisystemic genetic disorder with autosomal dominant inheritance, characterized by hamartomas that affect multiple organs, including the central nervous system, skin, heart, kidneys, and lungs. It occurs due to variants in the tumor suppressor genes TSC1 or TSC2. In this case series, we present a subgroup of 7 pediatric patients with variants in the TSC2 gene and discuss their genotypes, phenotypes as well as genotype-phenotype correlation.

Keywords: Tuberous sclerosis complex, Tuberous sclerosis, TSC2, Pediatrics

INTRODUCTION

Tuberous sclerosis complex (TSC) is a multisystemic genetic disorder with autosomal dominant inheritance, characterized by hamartomas that affect multiple organs, including the central nervous system, skin, heart, lungs, and kidneys.[1] It occurs due to variants in the tumor suppressor genes TSC1 or TSC2, which lead to aberrant proteins hamartin and tuberin, codified in the loci 9p34 and 16p13, respectively.[2,3] The complex hamartin/tuberin regulates cell growth via the phosphatidylinositol 3-kinase signaling pathway by suppressing the mammalian target of rapamycin (mTOR), responsible for the proliferation and inhibition of apoptosis. Therefore, changes in these proteins result in persistent activation of the mTOR pathway and, as a result, the creation of multisystemic hamartomas.[4] Genetic testing identifies no mutation in approximately 10-25% of TSC patients; therefore, a normal result does not exclude the diagnosis.[1] TSC affects one in every 6,000 to 10,000 individuals and can affect both sexes and all ethnic groups equally.[5]

In this case series, we present a subgroup of 7 pediatric patients with variants in the TSC2 gene and discuss their genotypes, phenotypes as well as genotype-phenotype correlation.

CASE SERIES

This study enrolled 7 pediatric patients. Patients #2 and #3 are sisters. Variant coordinates are after the human genome variation society (HGVS) nomenclature as provided by the laboratory reports. The classes of the variants are defined according to the standards and guidelines for the interpretation of sequence variants of the American college of medical genetics and genomics (AMCG) and the association for molecular pathology (AMP). The clinical information (phenotype) indicated below follows the human phenotype ontology (HPO) nomenclature. Table 1 presents genetic data of patients.

Patient 1

Patient 1 is an 18-year-old girl that presented adenoma sebaceum, anemia, astrocytoma, ataxia, cerebral calcification, cortical tubers, headache, hepatic calcification, hypomelanotic macule, hyperreflexia, hypertonia, nephrocalcinosis, shagreen patch, spasticity, seizures, strabismus, and vomiting. The age at onset was

8 months. A pathogenic (class 1) heterozygous de novo variant with coordinates c.1832G>A in the TSC2 gene was identified by whole exome sequencing (WES).

Patient 2

Patient 2 is a 15-year-old girl that presented with abnormal cortical gyration, ataxia, behavioral abnormality, developmental regression, dystonia, encephalopathy, hyperreflexia, hypertonia, intellectual disability, and seizures. The age at onset was 7 months. A heterozygous variant of uncertain significance (class 3), paternally inherited, with coordinates c.1599+5G>C in the TSC2 gene was identified by WES.

Patient 3

Patient 3 is a 17-year-old girl, the sister of patient 2, that presented only seizures, and a shagreen patch. The age at onset was 18 months. The variant is identical to the patient 2.

Patient 4

Patient 4 is a 13-year-old girl that presented abnormal cortical gyration, abnormal renal morphology, ataxia, autism, delayed speech and language development, dystonia, encephalopathy, hyperreflexia, hypertonia, hypomelanotic macule, intellectual disability, intracerebral periventricular calcifications, microcephaly, motor delay, seizure, and spasticity. The age at onset was 13 months. A likely pathogenic (class 2), heterozygous, maternally inherited variant with coordinates c.1547dup in the TSC2 gene was identified by WES.

Patient 5

Patient 5 is a 10-year-old girl that presented ataxia, brain dysplasia, delayed speech and language development, hyperreflexia, hypomelanotic macule, intellectual disability, motor delay, and seizures. The age at onset was 10 months. A likely pathogenic (class 2), heterozygous, de novo variant with coordinates c.87_94del in the TSC2 gene was identified by WES.

Patient 6

Patient 6 is a 4-year-old girl that presented abnormal myelination, hypomelanotic macule, intellectual disability, and seizures. The age at onset was 8 months. A pathogenic (class 1), heterozygous, paternally inherited variant with coordinates c.2355+2_2355+5del in the TSC2 gene was identified by WES.

Patient 7

Patient 7 is a 2-year-old boy that presented hypomelanotic macule, hypotonia, intracerebral periventricular calcifications, and seizures. The age at onset was 9 months. A pathogenic (class 1) heterozygous, maternally inherited variant with coordinates c.5068+1G>C in the TSC2 gene was identified by WES.

Table 1: Genetic data.

No./sex	Variant coordinates in the TSC2 gene	Amino acid change	Status of variant	Variant type	Disease-associated mechanism	Class. ACMG
1/ F	c.1832G>A	p.(Arg611Gln)	De novo	Substitution	Missense	class 1
2/ F	c.1599+5G>C	-	Paternally inherited	Substitution	-	class 3
3 /F	c.1599+5G>C	-	Paternally inherited	Substitution	-	class 3
4 /F	c.1547dup	p.(Glu517Argfs*72)	Maternally inherited	Duplication	Frameshift	class 2
5/ F	c.87_94del	p.(Ser30Glyfs*2)	De novo	Deletion	Frameshift	class 2
6/ F	c.2355+2_2355+5del	-	Paternally inherited	Deletion	Splicing	class 1
7/ M	c.5068+1G>C	-	Maternally inherited	Substitution	Splicing	class 1

Class. ACMG=classification according to the American college of medical genetics and genomics; F=female; M=male; No.=number; TSC2=Tuberous sclerosis complex type 2; -=unknown.

DISCUSSION

Genotype

According to the second international TSC consensus conference held in 2012, the presence of a pathogenic variant in either TSC1 or TSC2 gene is considered an independent diagnostic criterion, sufficient to confirm the diagnosis of tuberous sclerosis. Pathogenic is defined as "a mutation that clearly prevents protein synthesis and/or inactivates the function of the TSC1 or TSC2 proteins e.g., nonsense or frameshift mutations, or large genomic deletions) or a missense mutation whose effect on protein function has been established by functional assessment". Other variants whose effect on function is less certain are not sufficient to make a definite diagnosis of TSC.[1]

All the variants in our patients were identified by whole exome sequencing in the TSC2 gene.

Patient #1 variant causes an amino acid change from arginine to glutamine at position 611, thus the predicted amino acid change is p.(Arg611Gln). Its disease-associated mechanism is missense. This variant is previously described by Au et al, Nellist et al and Milunsky et al.[6-8] Patient #2 and #3 identical variants are predicted to disrupt the highly conserved donor splice site of intron 15. The disease-associated mechanism and predicted amino acid change are unknown. Patient #4 variant creates a shift in the reading frame starting at codon 517, the new reading frame ends in a stop codon 71 positions downstream. Its disease-associated mechanism is hence frameshift. The predicted amino acid change is p.(Glu517Argfs*72). Patient #5 variant creates a shift in the reading frame starting at codon 30, the new reading frame ends in a stop codon 1 position downstream. Its disease-associated mechanism is frameshift as well. The predicted amino acid change is p.(Ser30Glyfs*2). Patient #6 variant is in close proximity to the highly conserved splice site of intron 21. It is previously described by Niida et al, Le Caignec et al and Rosset et al.[9-11] Patient #7 variant is predicted to disrupt the highly conserved donor splice site. The disease-associated mechanism for both patients #6 and #7 is splicing, while the respective predicted the amino acid changes are not known.

Based solely on the genetic test results and the updated diagnostic criteria of 2012, patients #1, #4, #5, #6 and #7 have a definite diagnosis of TSC type 2 meanwhile, patients #2 and #3 have a possible diagnosis.

Phenotype

In the medical literature, TSC presents a wide clinical spectrum, as witnessed by our patients. Due to phenotypic diversity, diagnosis of TSC based only on phenotype can sometimes be difficult, even among individuals from the same family who carry the same disease-causing variant, like patients #2 and #3.

The clinical diagnostic criteria updated in the second international TSC consensus conference in 2012 include a no. of major and minor features, as in Table 2.[1]

Based solely on the clinical information and the updated clinical diagnostic criteria of 2012, patients #1, #4, and #5 have a definite diagnosis of TSC type 2, whereas, the remaining patients have a possible diagnosis.

Table 2: Updated clinical diagnostic criteria for TSC, 2012.

Criteria	
Major criteria	Hypomelanotic macules (≥3, at least 5 mm diameter)
	Angiofibromas (≥3) or fibrous cephalic plaque
	Shagreen patch
	Ungual fibromas (≥2)
	Cortical dysplasias*
	Multiple retinal hamartomas
	Subependymal nodules
	Subependymal giant cell astrocytoma
	Cardiac rhabdomyoma
	Lymphangioleiomyomatosis (LAM)**
	Angiomyolipomas (≥2)**
Minor criteria	Dental enamel pits (>3)
	Intraoral fibromas (≥2)
	Nonrenal hamartomas
	Multiple renal cysts
	Retinal achromic patch
	"Confetti" skin lesions
Definite diagnosis	2 major features or 1 major + 2 minor features
Possible diagnosis	either 1 major feature or 2 minor features

* Includes tubers and cerebral white matter radial migration lines.
**A combination of LAM and angiomyolipomas without other features does not meet the criteria for a definite diagnosis.

Genotype-phenotype correlation

Genetic testing makes TSC diagnosis less vague, however, it cannot predict exact phenotype, severity of the disease, or its course, thus, genetic test results must be evaluated in the context of clinical findings, the family history, and other laboratory data.

According to large-scale studies comparing phenotypes in TSC2 versus TSC1 variants to find a correlation, features including cortical tubers, seizures, intellectual disability, Autism specter disorder, attention deficit hyperactivity disorder, psychiatric conditions, mental retardation, cardiac rhabdomyomas, renal angiomyolipoma and cysts, retinal phakomas, facial angiofibromas, subependymal

nodules are more frequently associated with variants in the TSC2 gene.[12,13]

In closing, considering both genetic and clinical diagnostic criteria, patients #1, #4, #5, #6, and #7 have a definite diagnosis of TSC type 2, while patients #2 and #3 (sisters) with only one major clinical feature and a variant of uncertain significance (class 3) because of the unknown effect on the tuberin protein, have a possible diagnosis of TSC type 2.

CONCLUSION

Tuberous sclerosis complex is a multisystemic genetic disorder with wide phenotypic variability, challenging to diagnose based solely on genetic testing or clinical presentation; therefore, conversely, it is critical to assess genetic test results in the context of clinical findings, family history, or other data. Individual phenotype prediction and genotype-phenotype correlation remain elusive, despite ongoing advances in genetic testing technologies.

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