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CASE REPORT

Clinical and genetic analysis of patients with segmental overgrowth features and somatic mammalian target of rapamycin (mTOR) pathway disruption: Possible novel clinical issues

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Abstract

Segmental overgrowth syndromes include a group of clinical entities, all characterized by the abundant proliferation of tissues or organs in association with vascular abnormalities. These syndromes show a wide spectrum of severity ranging from limited involvement of only small areas of the body to complex cases with impressive distortions of multiple tissues and organs. It is now clear that somatic mutations in genes of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway (in brief "mTOR pathway") are responsible for such entities. Not all the cells of the body carry the same causative mutation, which is mosaic, appearing from two (or more) distinct cell lineages after fertilization. In this article, we reconsider the clinical spectrum and surveillance programs of patients with segmental overgrowth syndromes, based on the features of six patients with diverse clinical forms of overgrowth and pathogenic variants in genes of the mTOR pathway.

KEYWORDS

genetic mosaicism, genotype–phenotype correlations, MTOR, PIK3CA, segmental overgrowth, somatic mutations

1 | INTRODUCTION

Segmental overgrowth syndromes include a group of diverse clinical pictures all characterized by the exaggerate proliferation of organs or tissues, mostly associated with vascular anomalies. Excessive growth may affects the adipose, skeletal, muscle, nervous, fibrous, vascular, or lymphatic tissues and can be accompanied

by skin abnormalities (including epidermal nevi, hyperpigmented or hypopigmented lesions; Eng et al., 2020).

In the last years, advanced genetic technologies like next generation sequencing (NGS) have dramatically improved the knowledge of molecular mechanisms underlying segmental overgrowth syndromes, demonstrating an important causative role for somatic mutations disrupting mammalian target of rapamycin (mTOR) pathway.

mTOR pathway is known for its fundamental role in many cellular processes such as growth, proliferation, angiogenesis, and metabolism. Activating mutations in any of these genes can lead to the impairment of cell cycle processes and induction of overgrowth, angiogenesis, and/or tumorigenesis (Keppler-Noreuil et al., 2016; Lindhurst et al., 2011; Mirzaa et al., 2014).

The pathogenesis of segmental overgrowth disorders consists of a postzygotic genetic mutation during embryogenesis in a population of somatic cells derived from the original zygote, resulting in two (or more) genetically distinct cell lineages. Somatic or germline involvement or both depends on the timing the variant occurs during embryogenesis. Mutations in somatic cells are typically de novo, not inherited from a parent. Mutations in the mTOR pathway genes involved in the pathogenesis of segmental overgrowth include *PIK3CA*, *MTOR*, *AKT*, *TSC1*, and *TSC2* and manifest as an autosomal dominant trait (Dazert and Hall, 2011; Laplante and Sabatini, 2012).

In this article, we present six different cases of segmental overgrowth associated with mutations of *PIK3CA* and *MTOR* genes (Table 1).

1.1 | *PIK3CA*-related overgrowth spectrum

Mosaic gain-of-function variants in the PIK3CA gene, encoding phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform, can determine different, sometimes overlapping, overgrowth patterns of a broad range of tissues. The principal targets of disease involvement include brain, limbs (possibily involving fingers and toes), trunk (abdomen and chest), and face, all usually with an asymmetric distribution. Unilateral involvement is typical too (Mirzaa et al., 2014). The clinical severity is highly variable with various different entities described. This may be dependent upon the timing of when the variant occurs during embryogenesis, the affected tissue, and the kind of pathogenic variant (Mirzaa et al., 2014).

PIK3CA overgrowth spectrum (PROS) includes diverse clinical issues, sometimes with a combination of the following signs and symptoms: megalencephaly-capillary malformation syndrome (MCAP), hemimegalencephaly (HMEG), focal cortical dysplasia (FCD), polymicrogyria (PMG), CLOVES syndrome, characterized by congenital lipomatous overgrowth (CLO), vascular malformations (V), epidermal nevi (E), and scoliosis/skeletal or spinal malformation (S), fibroadipose hyperplasia, hemihyperplasia, macrodactyly, infiltrating lipomatosis, and isolated tissue dysplasia-overgrowth phenotypes like lymphatic or vascular malformations on skin or in inner organs (Kurek et al., 2012).

Variable degrees of developmental delay, intellectual disability, hypotonia, feeding difficulties, and epilepsy are commonly described together with possible behavioral concerns and autistic spectrum disorder, while cortical brain anomalies or Chiari I malformation could be a neuroradiological finding. Skeletal and limb abnormalities include scoliosis, digital anomalies like macrodactyly, cutaneous syndactyly, or polydactyly (Mirzaa et al., 2014). Epidermal and dermal nevi, cutis marmorata, hypopigmented macules, and patchy hyperpigmentations following the lines of Blaschko are among the most frequent dermal manifestations of PROS. There is increasing evidence that somatic variants in PIK3CA gene may be the cause for different, benign, or malignant tumors (Dobyns and Mirzaa, 2019). It is worth noting that in a recent work by Peyre, somatic mutations in PIK3CA have also been identified in association with cerebral cavernous malformations (Peyre et al., 2021).

2 | METHODS

Six patients were recruited at IRCCS Gaslini Institute (Genoa, Italy) between years 2017 and 2021, selected and anonymized for this study.

Genomic DNA was isolated from 1 ml of peripheral blood using QIAamp® DNA Blood Midi (Qiagen). We assessed the concentration and the quality at 260/280 nm of the isolated DNA using the NanoDrop spectrophotometer (NanoDrop Technologies, Delaware).

MTOR (NM_004958, 58 exons, 8,677 bp), PIK3CA (NM_ 006218, 21 exons, 9,093 bp), PIK3R2 (NM_ 005027, 16 exons, 4,033 bp), CCND2 (NM_ 001759, 5 exons, 6,480 bp), TSC1 (NM_ 000368, 23 exons, 8,604 bp), TSC2 (NM_ 000548, 42 exons, 6,156 bp), AKT3 (NM_ 005465, 13 exons, 5,127 bp), PTEN (NM_ 000314, 9 exons, 9,027 bp) amplicons were designed using the AmpliSeq Designer Software v2.0 (Life Technologies) targeting the coding sequences and 10 bases of the adjacent intron regions; the design resulted in a total of 217 amplicons divided in two multiplex primer pools.

Amplicon library was prepared using the Ion AmpliSeq Library Kit 2.0 (Life Technologies): primer pools

TABLE 1 The main clinical, radiological, and genetic issues identifiable in our cohort

	P1	P2	P3	P4	P5	P6
Sex	M	F	F	F	M	M
Macrocephaly	Yes	No	No	Yes	Yes	Yes
Cardinal CNS anomalies	No	No	ND	PMG, corpus callosum hypoplasia	HMEG, FCD	HMEG
Associated CNS anomalies	No	Vascular malformation	ND	Chiari I malformation, hydrocephalus, low-lying conus medullaris	No	Chiari I malformation, hydrocephalus
Overgrowth pattern (extra-CNS)	Metacarpal asymmetry	Lymphoangiomatous dysplasia, heterometry fibroadipose lesions	Macrodactyly of toe	Hemihypertrophy, heterometry	No	Hemihypertrophy
Developmental delay	No	No	No	Yes	Yes	Yes
Tumors	Angiolipoma	Ovarian dysgerminoma, papillary thyroid carcinoma, neurofibromas	No	Suspect teratoma	No	No
Variant	c.3073A > G (p.Thr1025Ala)	c.1633G > A (p.Glu545Lys)	c.3140A > T (p.His1047Leu)	c.311C > T (p.Pro104Leu)	c.4448G > A (p.Cys1483Tyr)	c.3139C > T (p.His1047Tyr)
Gene	PIK3CA	PIK3CA	PIK3CA	PIK3CA	MTOR	PIK3CA
Detected on						
Blood	No	No	No	Yes	No	No
Skin fibroblasts	No	Yes	Yes	ND	ND	ND
Bioptic tissue	Yes (angiolipoma)	ND	ND	ND	Yes (brain)	ND
Oral epithelium	ND	No	ND	ND	ND	Yes
Additive features	No	Scoliosis, hypergonadotropic hypogonadism, colonic diverticulum, and cystic hygroma	No	No	Seizures	Dermal angiomas and skin dyschromias

Abbreviations: CNS, central nervous system; FCD, focal cortical dysplasia; HMEG, hemimegalencephaly; ND, not determined; P, patient; PMG, polymicrogyria.

were added to 10 ng of gDNA and amplified by PCR. The amplicons were ligated to the sequencing adapters, characterized by 20 different sequence-barcodes (Ion Xpress Barcode Adapters, Life Technologies) to allow sample assignment after pooling. The library was purified using the AgencourtAMPure XP Reagent (Beckmann Coulter, California). The concentration of the final library was determined by fluorescent measurement on Qubit 2.0 instrument (Life Technologies). Libraries from 20 different samples were diluted to 15 ng/ml and pooled together.

Template preparation was performed with Ion OneTouch kit v2 (Life Technologies) on Ion OneTouch2 instrument (Life Technologies) using an emulsion PCR method based on Ion Sphere Particle (ISP). The nontemplated ISPs were removed during the semiautomated enrichment process on Ion ES instrument (Life Technologies). After adding the sequencing primer and polymerase (Ion PGM 200 Sequencing kit v2, Life Technologies) the fully prepared ISPs were loaded into an Ion 314 chip, and the sequencing runs were performed with 500 flows on the Ion Torrent PGM machine (Life Technologies).

The sequence reads were analyzed using the Ion Reporter pipeline (Life Technologies) and the CLC Genomics Workbanch 6.5.1 software (Qiagen) for filtering out poor quality reads, alignment on hg19 reference, variant detection, and coverage analysis. Variants call was performed with a minimum coverage of 20 reads (≥20×). In some selected cases, low-frequency pipeline was lanched for identification of mosaic variants.

All the called variants were validated by Sanger sequencing using High-Fidelity Platinum Master Mix (Invitrogen) for PCR and BigDye Terminator v1.1 kit (Life Technologies) for sequencing.

The variant-predicted functional effect was assessed by Polyphen 2.0 for missense mutations and by Mutation Taster software for synonymous, exonic, or intronic variants that may cause splicing alteration.

3 | CLINICAL CASES

3.1 | Patient 1

Patient 1 is a male child, second-born of an Italian non consanguineous couple. His father presented with an in situ melanoma, asymmetric hands, and macrocephaly.

He was born after an uneventful pregnancy at 39 weeks of gestation by cesarean section because of a maternal uterine myoma. Weight was 3,300 g and length 51 cm. At birth, he had swelling in the nuchal region, consistent with an angiolipoma. Magnetic resonance imaging (MRI) of the area showed thickening of the adipose tissue in the nuchal area with caudal extension to the first thoracic vertebra (D1) with tortuous vessels in this context, suggestive of a vascular malformation. The child had normal psychomotor development and normal facial features. He showed macrocephaly and metacarpal asymmetry with the right hand wider than the left one.

Mutational screening of genes of the MTOR pathway was performed on the pathological tissue, revealing the somatic missense c.3073A > G (p.Thr1025Ala) variant in the PIK3CA gene, with an allele frequency of 25%. This variant, that involves a conserved amino acid of the protein, is present in ClinVar database and in the International Register of mutations, in association with MCAP. Based on these elements the variant was classified as pathogenic. The same somatic variant was previously reported (Medical Genetics Laboratory of Aldo Moro University- Bari, on June 7, 2021) as consistent with CLOVES syndrome (ClinVar). The *PIK3CA* c.3073G > A variant was not detected in patient's blood DNA nor in fibroblasts from the larger hand's skin. Segregation analysis on the father's skin fibroblasts could not identify the variant.

From this analysis we could infer that the same variant in *PIK3CA* can be associated with different clinical phenotypes, so a precise genotype–phenotype correlation remains elusive.

3.2 | Patient 2

Patient 2 is now a young woman, first-born of an Italian non consanguineous couple. Her brother has Ewing sarcoma, her mother has colonic polyps, and her aunt has thyroid cancer and breast fibroadenoma.

At birth, the patient showed impressive hypertrophy of the lower limbs, a cystic hygroma in the inguinal region, and an abdominal angiomatous lesion with thickening of the soft tissues. Abdominal ultrasounds showed the left kidney wider and on a more cranial position than the other. MRI of the abdomen and lower limbs showed features of lympho-angiomatous dysplasia involving the right leg, the abdominal wall, the left area of the pelvis, and the left kidney. Lower limbs asymmetry with disproportionately long feet with toes of different lengths was evident. Two surgical interventions of partial amputation of the feet became necessary.

At 13 years old the girl was diagnosed with ovarian dysgerminoma, which needed surgical excision and chemotherapy. At 14 years old she underwent surgery due to a worsening scoliosis. At 16 years old the girl was discovered to suffer from hypergonadotropic hypogonadism. At 18 years old the patient required thyroidectomy due to papillary carcinoma. Later on brain MRI showed an area

of hyperintensity with contrast enhancement at the left frontal bone, consistent with a cavernous angioma or a meningo-angiomatous hamartoma. At 19 years old a cystic/angiomatous lesion in the right kidney, a left ureteral dilatation, and a spleen cyst were found. A colonic diverticulum could be appreciated. At 24 the girl was diagnosed with dorsal and subcostal neurofibromas, needing surgical excision.

Clinically the girl showed a few facial dysmorphisms: a long face, downslanted palpebral fissures, thin and curved nose, micrognathia, arched palate, and dental fragility, abdominal hemangiomas, abdominal and inguinal lipomas, asymmetric lower limbs with subcutaneous thickening, big feet with toes of different length and size, skin folds, and recurrent infections of toes.

Despite the absence of cerebriform connective tissue nevi and palmoplantar keratoderma, in suspicion of Proteus syndrome, an analysis of AKT1 in skin biopsy was negative and analysis of PTEN was normal. The girl received a clinical diagnosis of CLOVES syndrome, and her venous-lymphatic dysplasia worsened over time.

A molecular analysis for mTOR pathway genes was performed both on DNA from peripheral blood and oral brushed epithelium, identifying no mutations.

Finally, the analysis performed on biopsy of the skin, overlying a dorsal neurofibroma revealed the somatic pathogenic variant c.1633G > A (p.Glu545Lys) (allele frequency = 47%) in the *PIK3CA* gene, associated with CLOVES syndrome, MCAP and other PROSs (ClinVar).

Even if the patient's phenotype recalls the CLOVES syndrome, some features appear not to be classically reported like ovarian dysgerminoma, papillary thyroid carcinoma, colonic diverticulum, multiple neurofibromas, and hypogonadism. Moreover, the patient lacked epidermal nevi on her body. To date, it is difficult to establish whether these clinical manifestations are attributable to the somatic mutation identified, but an association cannot be excluded.

3.3 | Patient 3

Patient 3 is a girl, second-born of an Italian nonconsanguineous couple. The sister has recently received a diagnosis of hystiocytosis of the cranial theca. A first cousin suffers from epilepsy, two second-degree cousins, respectively show mild cognitive impairment and seizures. In the father's family, a second degree cousin died at 40 due to lung cancer.

Pregnancy was affected by hypochromic anemia. The girl was born at 39 weeks and 5 days, by cesarean section due to fetal tachycardia. Birth weight was 3,420 g, length 49 cm, and head circumference 35 cm. APGAR scores were 9 and 10 at 1 and 5 min, and the child had normal development. At 6 months an abnormal and asymmetrical growth of the first toe of the left foot started to become evident in association with the thickening of subcutaneous tissue (Figure 1a). X-rays of the left foot showed dysmorphism of the distal phalanx of the hallux with a widened and stocky apex with hyperplasia of the soft tissues. Abdominal x-rays were negative. The child has bilateral single transverse palmar flexion creases.

Comparative Genomic Hybridization (CGH)-array analysis and NGS analysis of mTOR pathway genes on blood DNA were negative but we could identify the somatic variant c.3140A > T (p.His1047Leu) in *PIK3CA* in DNA from skin fibroblast with an allele frequency of 57% and involving an evolutionary conserved aminoacid of the protein, classified as pathogenic.

This somatic variant is already described in ClinVar, in association with different other phenotypes. The child's phenotype is consistent with isolated macrodactyly of toes.

3.4 | Patient 4

Patient 4 is a female child, second-born of an Italian nonconsanguineous couple with a noncontributive family history. She was born at 37 weeks by cesarean section because of failure to progress. Weight at birth was 3,330 g, length 49 cm, and head circumference 38.5 cm (+4.07 SDS). During the first day of life she was transferred to the Neonatal Pathology Unit because of hypothermia, cyanosis and hypocalcemia. Macrocephaly was accompanied by Chiari I malformation, hemihypertrophy, facial dysmorphisms, skin, and vascular abnormalities. Later on, developmental and language delay became evident. Head MRI showed wide lateral ventricles, a triventricular hydrocephalus, a smaller posterior fossa with vertical cerebellar tentorium. Cerebellar tonsils were mildly protruding through the foramen magnum, with crowding at the occipital-cervical junction. The right sylvian fissure appeared to be asymmetric and deeper than the contralateral and showed thick cerebral cortex, with PMG. A mild hypoplasia of the corpus callosum was present (Figure 1d,e). Sigmoid sinuses and jugular veins aplasia with posterior venous reflux were appreciated. A later brain and spinal MRI showed hydrocephalus and a low-lying conus medullaris at L3 level.

Regarding the dermatologic evaluation livedo reticularis, superior philtrum angioma, cutis marmorata with acrocyanosis of hands and feet were present. The child also appeared to have a low nasal bridge, hypotonia, and clitoral hypertrophy. Heart ultrasound revealed hypoplasia of the inferior vena cava, a 3 mm superior vena cava

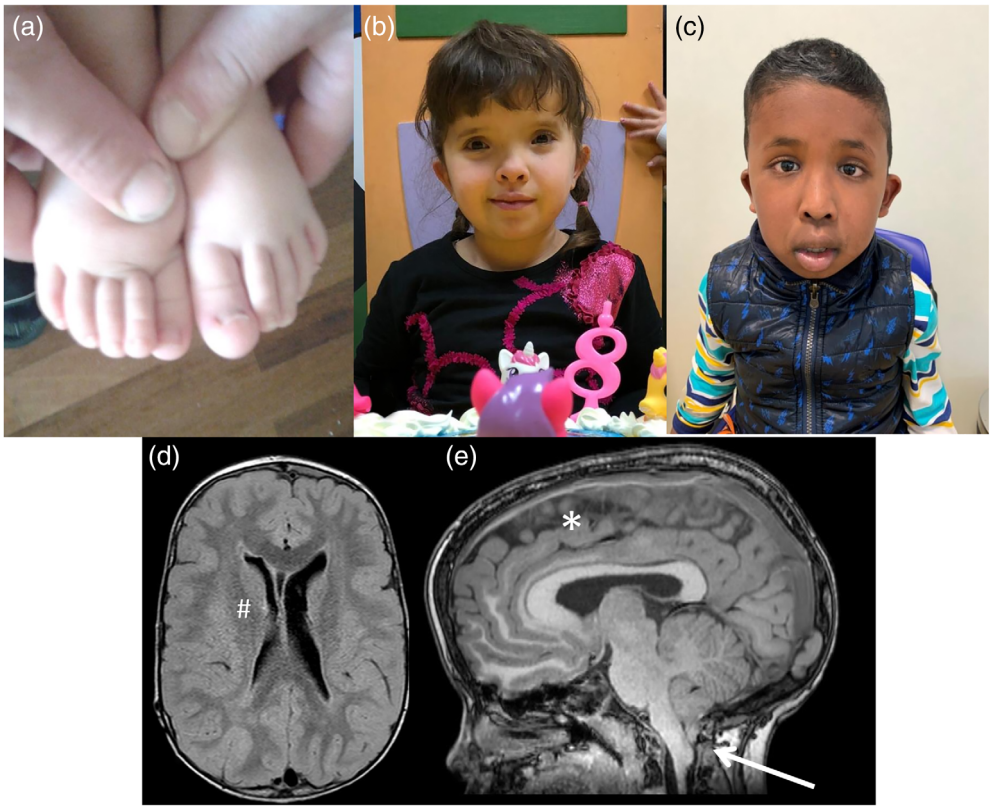

FIGURE 1 Patterns of segmental overgrowth and dysmorphisms in our patients' cohort; (a) Patient 3: macrodactyly of the left toe; (b) Patient 4: macrocephaly, hypertelorism, asymmetric eyes, blepharophymosis, angioma on the philtrum, low-set ears; (c) Patient 6: frontal bossing, sparse eyebrows, deep-set eyes and long palpebral fissures, strabismus, prominent philtrum, thick lips with a "whistling mouth" appearance; (d,e) Brain MRI of Patient 4 showing: Chiari I malformation with a mild protrusion of cerebellar tonsils through the foramen magnum (arrow), asymmetric widening of the lateral ventricles (hash), a triventricular hydrocephalus, a smaller posterior fossa with vertical cerebellar tentorium, hemispheric asymmetry and areas of polymicrogyria (asterisk), mild hypoplasia of the central part of the corpus callosum

with an increased diameter of the azygos vein. A later brain MRI showed an increased stenosis of the foramen magnum and malformations suggestive of MCAP.

Later on, abdominal ultrasound revealed a small round formation within the left ovary, with an inhomogeneous echoic appearance, consistent with teratoma. Moreover, it was possible to notice an increased level of the tumoral markers alpha-fetoprotein and CA125.

Recently the child underwent a new genetic examination in which clumsiness and mild cognitive impairment were more evident. Dysmorphic features like lower limb asimmetry (right > left), exophoria, hypertelorism with blepharophimosis, low-set and posteriorly angulated ears, geographic tongue, and carious teeth were identifiable (Figure 1b). New measurements revealed a head circumference corresponding to +7.3 SDS. Diagnostic investigations about the ovarian lesion are being in progress.

Karyotype and CGH-array were negative. The molecular analysis of *AKT3* and *PIK3CA* genes on leucocytes DNA identified a mosaic pathogenic variant in the latter. The heterozygous c.311C > T, (p.Pro104Leu) variant in *PIK3CA* was present in 32% of the examined cells. The variant is defined as likely pathogenic in ClinVar in association with PROS, which correlates with the clinical picture of the patient.

3.5 | Patient 5

Patient 5 is an only child of an Italian, nonconsanguineous couple. The mother was reported with ulcerative colitis. In the father's family a first cousin was referred with mild cognitive impairment and aggressive behavior; the grandfather was said to have had bilateral eighth cranial nerve neurinoma.

Pregnancy was uneventful except for uterine contractions at 36 weeks. Delivery was spontaneous at 39 weeks with the following parameters: weight 3,290 g, length 50 cm, and head circumference 35 cm.

At 5 months of age the child showed eye deviation associated with right arm hyperextension. Electroencephalogram (EEG) identified hypsarrhythmia and, in suspicion of West Syndrome, a therapy with adrenocorticotropic hormone (ACTH) and Vigabatrin was started. Eyes deviation is still episodically present. The child also presented with developmental delay. Head MRI revealed right HMEG. During the first clinical genetic evaluation his head circumference corresponded to the 76th pc. The baby was irritable and had fluctuating limb hypertonia. A salivary genetic testing in search for mTOR pathway genes' variants resulted negative.

The child underwent hemispheric disconnection due to severe, nonresponsive epilepsy. The analysis of mTOR pathway genes on brain tissue revealed the c.4448G > A (p.Cys1483Tyr) variant of the *MTOR* gene in 15–20% of cells. This variant is reported in literature in patients with HMEG and FCD. It introduces an aminoacid change, resulting in a functional effect on the protein. The same variant was not present in peripheral blood.

This variant is classified as pathogenic in ClinVar, described in association with mTOR-related hypomelanosis of Ito with neurodevelopmental abnormalities. In a paper by Lee et al. (2012), the same variant is reported in association with HMEG, in analogy with the clinical picture of our patient, who however lacked hypomelanosis.

3.6 | Patient 6

Patient 6 was born to a nonconsanguineous couple of Bengali origin. Family history was not remarkable. The child's phenotype was characterized by cognitive impairment, macrocephaly, right HMEG, hydrocephalus, mild Chiari I malformation, right hemihypertrophy, multiple skin dyschromias, and three café-au-lait spots with cutaneous angiomas. Head circumference and height were + 3.11 and + 2.94 SDS, respectively. Other dysmorphisms were observed such as frontal bossing, sparse eyebrows, deep-set eyes and long palpebral fissures, strabismus, prominent philtrum, thick lips with a "whistling mouth" appearance, sandal gap, thickening of the subcutaneous tissue of hands and feet, and hallux bilaterally shorter than the second toe (Figure 1c).

The study of mTOR pathway genes on peripheral blood and CGH-array analysis were normal. Genetic screening using DNA of oral epithelium identified a somatic pathogenic variant in the *PIK3CA* gene. The c.3139C > T (p.His1047Tyr) mutation involves a conserved aminoacid and is predicted to have functional consequences on the protein. The variant is recognized as pathogenic in ClinVar. It had been previously reported in HMEG, MCAP syndrome, CLO, vascular malformations, and epidermal nevi and in capillary malformation of the lower lip, lymphatic malformation of face and neck, asymmetry of face and limbs, and partial/generalized overgrowth (ClinVar).

The patient's phenotype is consistent with the clinical signs associated with the variant, except for PMG which is not recognizable in this case.

4 | DISCUSSION

In this article, we provide a detailed clinical description of six patients with segmental overgrowth syndrome associated with mutations, mainly somatic, of the *PIK3CA* or *MTOR* genes. The variants detected in our patients are classified as "pathogenic," "likely pathogenic," (Patient 4) and "pathogenic/likely pathogenic" (Patient 2). All mutations are just described in literature, except for the PIK3CA c.311C > T (p.Pro104Leu) (Patient 4).

As the vast majority of *PIK3CA* and *MTOR* pathogenic variants arise during the postzygotic phases, the diagnostic testing may include genetic analysis of more than one tissue. In all cases, the pathogenic variant was detectable only by oral brushing, dermal, or pathologic tissue biopsy, except for Patient 4 whose pathogenic variant was detectable on blood. Nevertheless, failure to detect pathogenic variant in genes of MTOR pathway does not exclude a clinical diagnosis of a segmental overgrowth disorder in individuals with suggestive features (Mirzaa et al., 2013, Updated 2021).

Our patients showed the typical overgrowth patterns of mTOR pathway disruption: MEG is described in 2 out of 6 patients (1 and 4), HMEG in other 2 patients (5 and 6), CLOVES syndrome in 1 child (Patient 2); limbs/toes overgrowth, lipomatosis, and vascular malformations are all identifiable in our cohort, to a variable degree. As regards possible neurologic issues involved in these syndromes, cognitive impairment is present in 3 out of 6 cases (Patients 4, 5, and 6), while seizures and hypotonia are described only in one patient respectively (Patients 5 and 4).

Despite the classical phenotypes observed in our cohort, additionally hypergonadotropic hypogonadism, colonic diverticulum, multiple neurofibromas, ovarian dysgerminoma, and papillary thyroid carcinoma were described in Patient 2, and not previously reported. Patient 5 lacked Ito hypomelanosis which had been previously reported in another patient with overgrowth and Ito hypomelanosis, harboring the same genetic variant. Endocrinology issues are rarely reported in PROS patients, including hypoglycemia, growth hormone deficiency, or other forms of hypothalamic–pituitary dysfunction (Douzgou et al., 2022); however, to date no case of

peripheral hypogonadism, as in Patient 2, is found in the literature.

Remarkable is the presence of Chiari I malformation in Patients 4 and 6. In particular Patient 4 showed it in association with PMG, areas of thick cerebral cortex, corpus callosum hypoplasia, and a low-lying conus medullaris. These findings together with the "cardinal signs" of MEG, HMEG, or PMG, suggest the importance of neuroimaging studies for a better dissection of the phenotype and prevention of possible neurological symptoms (Conway et al., 2007; Rivière et al., 2012).

The study of our cohort also highlights the presence of multiple benign and malignant tumors. Patient 2 was respectively diagnosed with papillary thyroid carcinoma, subcostal neurofibromas and ovarian dysgerminoma whereas Patient 4 was observed with ovarian teratoma. These findings appear to be in line with the well-known potential carcinogenetic role of somatic mutations in *PIK3CA* and *MTOR* genes.

Several attempts of establishing an "ad-hoc" genotype–phenotype correlation in MTOR pathway disruption have been made so far (Mirzaa et al., 2016; Zenner et al., 2019). However from our analysis, we can infer that a precise genotype–phenotype correlation is still difficult to establish since there is evidence that the same variant can produce highly different focal overgrowth patterns. Indeed Patient 1, affected by a typical MCAP syndrome, was carrying a variant that had been previously described in association with CLOVES syndrome. More data from future studies are required about genetic and/or environmental modifiers in order to deeply understand this correlation.

In conclusion, this work suggests the possible association with rarely/not previously described clinical items in patients with segmental overgrowth and the importance of deep, precise clinical, and neuroimaging evaluations. To date, a unique, precise surveillance program (especially for oncologic risk) is not available. Recently, an international expert consensus statement was made on the preferential approach for managing affected individuals based on current knowledge with the aim to create models for the sustainable care and management of PROS patients. This new approach is complementary to condition-specific guidelines (e.g., for patients with epilepsy). The need for some specific clinical aspects remains elusive, in particular it is not clear if a cancer prevention protocol is necessary for every patient with segmental overgrowth, given the absence of systematic data. For this reason, the authors recommend counseling the families on a case-by-case basis. Further longitudinal studies and clinical trials are necessary to provide precise management recommendations for these aspects (Douzgou et al., 2022).

As somatic genetic alterations in the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway predispose to cancer, considerable research and development efforts have been developed to generate inhibitors of PI3K, AKT, and mTOR. Several of these compounds are in clinical trials for cancer and may be re-purposed as targeted therapies for segmental overgrowth conditions.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Conway, R. L., Pressman, B. D., Dobyns, W. B., Danielpour, M., Lee, J., Sanchez-Lara, P. A., ... Graham, J. M., Jr. (2007). Neuroimaging findings in macrocephaly-capillary malformation: A longitudinal study of 17 patients. *American Journal of Medical Genetics. Part A*, *143A*(24), 2981–3008. https://doi.org/10.1002/ajmg.a.32040

Dazert, E., & Hall, M. N. (2011). mTOR signaling in disease. *Current Opinion in Cell Biology*, *23*(6), 744–755. https://doi.org/10.1016/j.ceb.2011.09.003

Dobyns, W. B., & Mirzaa, G. M. (2019). Megalencephaly syndromes associated with mutations of core components of the PI3K-AKT-MTOR pathway: PIK3CA, PIK3R2, AKT3, and MTOR. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *181*(4), 582–590. https://doi.org/10.1002/ajmg.c.31736

Douzgou, S., Rawson, M., Baselga, E., Danielpour, M., Faivre, L., Kashanian, A., ... Biesecker, L. G. (2022). A standard of care for individuals with PIK3CA-related disorders: An international expert consensus statement. *Clinical Genetics*, *101*(1), 32–47. https://doi.org/10.1111/cge.14027

Eng, W., Hammill, A. M., & Adams, D. M. (2020). Overgrowth syndromes and new therapies. *Seminars in Pediatric Surgery*, *29*(5), 150974. https://doi.org/10.1016/j.sempedsurg.2020.150974

Keppler-Noreuil, K. M., Parker, V. E., Darling, T. N., & Martinez-Agosto, J. A. (2016). Somatic overgrowth disorders of the PI3K/AKT/mTOR pathway & therapeutic strategies. *American Journal of Medical Genetics Part C of the Seminars in Medical Genetics*, *172*(4), 402–421. https://doi.org/10.1002/ajmg.c.31531

Kurek, K. C., Luks, V. L., & Ayturk, U. M. (2012). Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. *American Journal of Human Genetics*, *90*(6), 1108–1115.

Laplante, M., & Sabatini, D. M. (2012 Apr 13). mTOR signaling in growth control and disease. *Cell*, *149*(2), 274–293. https://doi.org/10.1016/j.cell.2012.03.017

Lee, J. H., Huynh, M., Silhavy, J. L., Kim, S., Dixon-Salazar, T., Heiberg, A., ... Gleeson, J. G. (2012). De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nature Genetics*, *44*, 941–945.

Lindhurst, M. J., Sapp, J. C., Teer, J. K., Johnston, J. J., Finn, E. M., ... Biesecker, L. G. (2011). A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *The New England Journal of Medicine*, *365*(7), 611–619. https://doi.org/10.1056/NEJMoa1104017

Mirzaa, G., Graham, J. M., Jr., & Keppler-Noreuil, K. (2013). PIK3CA-related overgrowth Spectrum. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews®*. Seattle (WA): University of Washington, Seattle Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK153722

Mirzaa, G., Parry, D. A., Fry, A. E., Giamanco, K. A., Schwartzentruber, J., Vanstone, M., ... Sheridan, E. G. (2014). De novo CCND2 mutations leading to stabilization of cyclin D2 cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome. *Nature Genetics*, *46*(5), 510–515. https://doi.org/10.1038/ng.2948

Mirzaa, G., Timms, A. E., Conti, V., Boyle, E. A., Girisha, K. M., Martin, B., ... Dobyns, W. B. (2016). *PIK3CA*-associated developmental disorders exhibit distinct classes of mutations with variable expression and tissue distribution. *JCI Insight*, *1*(9), e87623. https://doi.org/10.1172/jci.insight.87623

Peyre, M., Miyagishima, D., Bielle, F., Chapon, F., Sierant, M., Venot, Q., ... Kalamarides, M. (2021). Somatic *PIK3CA* mutations in sporadic cerebral cavernous malformations. *The New England Journal of Medicine*, *385*(11), 996–1004. https://doi.org/10.1056/NEJMoa2100440

Rivière, J. B., Mirzaa, G. M., O'Roak, B. J., Beddaoui, M., Alcantara, D., Conway, R. L., ... Dobyns, W. B. (2012). De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nature Genetics*, *44*(8), 934–940. https://doi.org/10.1038/ng.2331

Zenner, K., Cheng, C. V., Jensen, D. M., Timms, A. E., Shivaram, G., Bly, R., ... Bennett, J. T. (2019). Genotype correlates with clinical severity in PIK3CA-associated lymphatic malformations. *JCI Insight*, *4*(21), e129884. https://doi.org/10.1172/jci.insight.129884

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