Case Report

Phenotypic variability within *PIK3CA*-Related Overgrowth Spectrum, illustrated by two cases.

Willem Van Hoe [a], Hannelore De Maeseneer [b], Quentin Jordens [b], Dirk Van Gysel [b]

[a] Medical student, Faculty of Medicine, KU Leuven, Belgium
[b] Department of Pediatrics, O.L.Vrouw Hospital Aalst, Belgium

dirk.van.gysel@olvz-aalst.be

Keywords

PIK3CA gene; PIK3CA-Related Overgrowth Spectrum (PROS); CLAPO syndrome; Macrodactyly; somatic mosaicism

Abstract

PIK3CA-related overgrowth spectrum refers to a heterogeneous group of disorders caused by somatic pathogenic variants in the *PIK3CA* gene. Starting from two cases we recently diagnosed in our practice, 'Progressive Macrodactyly' and 'Capillary and Lymphatic malformation, Asymmetry of face and limbs, Partial or generalized Overgrowth', we illustrate the broad phenotypic variability possible within *PIK3CA*-related overgrowth spectrum. Current literature suggests the absence of a genotype-phenotype correlation. Further research is needed to establish a more adequate classification and a more accurate treatment.

Introduction

The term *PIK3CA*-related overgrowth spectrum (PROS) was first used by Keppler-Noreuil et al. to define a phenotypic spectrum of heterogeneous, rare entities, clinically characterized by asymmetric overgrowth and vascular malformations, and caused by somatic pathogenic variants in the gene *PIK3CA* (1,2).

Originally, PROS included the following syndromes with overlapping phenotypic features.

Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal Nevi, and Spinal abnormalities (CLOVES), Fibroadipose Hyperplasia or Overgrowth (FAO), Hemihyperplasia Multiple Lipomatosis (HHML), Dysplastic Megalencephaly (DMEG)/ Hemi Megalencephaly (HMEG), Megalencephaly-capillary Malformation (MCAP), Isolated Large Lymphatic Malformation (ILM), Facial Infiltrating Lipomatosis, Epidermal Naevi, Macrodactyly, Seborrheic Keratosis and Benign Lichenoid Keratosis (1,2).

However, over the years, multiple syndromes have been proposed to be included in PROS, such as Klippel-Trenaunay syndrome (proposed by Vahidnezhad in 2016) and CLAPO syndrome (Capillary and Lymphatic malformation, Asymmetry of face and limbs, Partial or generalized Overgrowth) (proposed by Rodriguez in 2018) (3,4).

The current identification of new syndromes linked to *PIK3CA* indicates the need for a reclassification in the near future when the spectrum of PROS will be more accurately documented and genotype-phenotype correlations will be better understood.

The *PIK3CA* gene encodes p110α, the catalytic subunit of phosphatidylinositide-3-kinase (PI3K) which is part of the complex PI3K-AKT-mTOR signaling pathway. PI3K catalyzes the conversion of phosphatidylinositol 4,5- bisphosphate (PIP2) to phosphatidylinositol 3, 4,5- trisphosphate (PIP3) which in its turn activates phosphoinositide-dependent kinase-1 (PDK1) which phosphorylates AKT (also known as protein kinase B). Activated AKT further phosphorylates downstream molecules like GSK-3, FoxO, and mTOR (figure 1).

This pathway plays a critical role in most cells by regulating biological processes such as cell proliferation and growth, apoptosis, metabolism and angiogenesis. Furthermore, it is linked to the pathogenesis of cerebrovascular and neurodegenerative diseases, diabetes mellitus and malignant tumours.

Heterozygous somatic gain-of-function variants in a mosaic pattern have been found in the above-mentioned PROS syndromes, explaining their clinical findings and the possible significant overlap (1,5,6).

In this article, we further illustrate the heterogeneity of PROS with two cases recently diagnosed in our department. The first case report describes an infant with a capillary malformation of the lower lip suggestive for CLAPO (7). In the second case report, a congenital enlargement of toes and later progressive enlargement of toes, foot and lower leg, in the context of Progressive Macrodactyly is described (8).

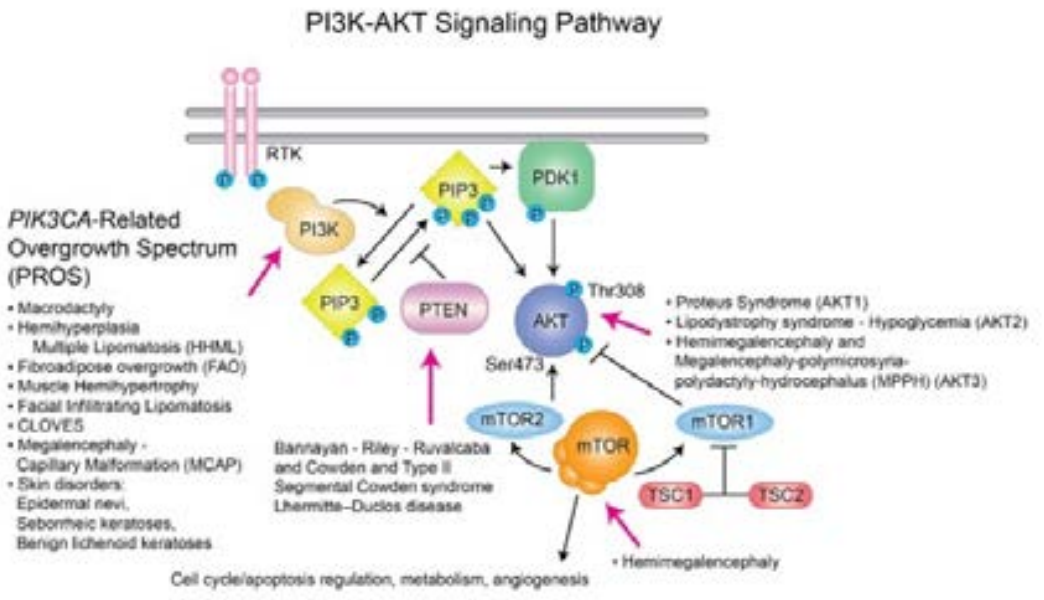
Case reports

The first case is a male newborn delivered after an uncomplicated pregnancy who presented at birth with skin lesions on the lower lip, face, head, and neck. Clinical examination showed a flat, red, blanchable, symmetrical zone on the lower lip with well-defined ragged edges that surrounded the entire lower lip vermillion and even surpassed it (figure 2). Additionally, a capillary malformation on the tip of the tongue was found, which is not shown on figure 2. Furthermore, diffuse reticulate patches of red to purple skin discoloration with underneath prominent veins located on the jaw, scalp, and neck were found (figure 3). No partial or generalized overgrowth was visible up to the age of 4 years. Psychomotor and neurological development were normal. No other vascular or cutaneous malformations were found on magnetic resonance imaging (MRI) of the head and neck. Further molecular investigation through skin biopsy was refused.

The final clinical diagnosis was CLAPO, a syndrome characterized by Capillary malformation of the lower lip, Lymphatic malformation of the face and neck, Asymmetry of the face and limbs, Partial or generalized Overgrowth. Overgrowth has been found only in a rare number of cases and is currently being doubted as clinical criterion. Lymphatic malformations can be present at birth, but may as well become evident after the neonatal period.

The second case is a girl who presented at birth with an enlargement of the first and second left toe (figure 4). The girl was born otherwise healthy after an uncomplicated vaginal delivery. Physical examination revealed a bigger size of the left foot compared to the right with a noticeable increase in circumference and length of the first and second toe. Conventional radiography and MRI of the left foot showed cortical thickening and an increased amount of fibrofatty tissue around the phalanges of the first and second toe. Histopathological examination of a biopsy specimen of the dermis and hypodermis confirmed the presence of abundant fatty tissue infiltrating the dermal connective tissue. Further genetic screening for *PIK3CA* variants was performed on cultured fibroblasts using Next Generation Sequencing on a MiSeq platform with two

Figure 1: *PI3K-AKT Pathway and associated clinical overgrowth disorders. Reprinted with permission from Keppler-Noreuil K, Rios J, Parker V, Semple R, Lindhurst M, Sapp J, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A. 2015;167A(2):287-95.*



panels, Tumor Hotspot MASTR Plus (Multiplicom) and Somatic 2 MASTR Plus (Multiplicom). A heterozygote c.1624G>A (p.Glu542Lys) missense variant in the *PIK3CA* gene was found in 69% of the cells of the affected tissue.

During the next months a further enlargement of the involved toes and foot was noticeable, and unfortunately also of the ipsilateral lower limb (figure 5). For this reason, an off-label treatment with sirolimus was started which halted further asymmetric overgrowth. The effect of sirolimus is being monitored by repeated measurements of the circumference of the feet, lower legs, knees, and upper legs, and the length of both lower legs. At the age of two, the aim is to switch sirolimus to a *PIK3CA*-inhibitor, for example BYL719.

These findings supported the diagnosis of a progressive form of Macrodactyly which is thus part of the *PIK3CA*-related overgrowth spectrum (PROS).

Discussion

In this paper, we describe two cases, respectively diagnosed as CLAPO and Progressive Macrodactyly, that belong to the *PIK3CA*-related overgrowth spectrum. The most common gain-of-function variants in the *PIK3CA* gene are the p.His1047Arg (found in 54% of cases), p.His1047Leu ((23%), p.Glu545Lys in (11%), p.Glu542Lys in (8%), and p.Cys420Arg (3%) (1).

However, multiple studies highlight the heterogeneity in variants found in multiple clinical phenotypes, supporting the absence of a genotype-phenotype correlation and so supporting the concept of *PIK3CA*-related syndromes as a single spectrum (1,6).

Keppler-Noreuil et al. hinted the possibility of a genotype-phenotype correlation with p.His1047Arg and p.His1047Leu as most common variants found in Macrodactyly cases. In our case of Progressive Macrodactyly, a p.Glu542Lys variant was found, providing further, even if limited, evidence for the lack of a genotype-phenotype correlation.

PROS needs to be suspected if early childhood-onset overgrowth, sporadic occurrence, and mosaic distribution are present. Vascular malformations and an epidermal naevus are also common findings. Patients suspected of having PROS should be tested for *PIK3CA* variants using a biopsy sample of the affected tissue. However, *PIK3CA* variants are sometimes also detectable in blood and buccal samples of patients with MCAP. Nonetheless, skin and other affected tissues have higher diagnostic rates and are therefore recommended as primary sample for diagnosis of all PROS phenotypes. Not finding any variants doesn't necessarily rule out PROS due to the possibility of accidental low-quality biopsies from non-affected regions. Multiple assays for testing PIK3CA variants are available with ultradeep next generation sequencing

Figure 2: *case 1 showing a flat, red, blanchable, symmetrical zone with well-defined edges that surround and surpass the entire lower lip vermillion.*

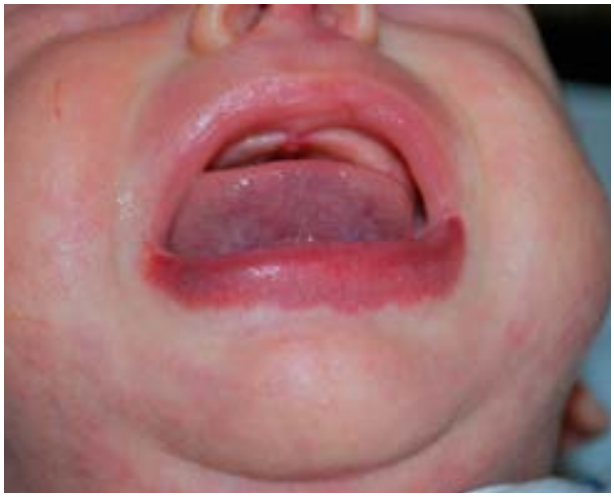
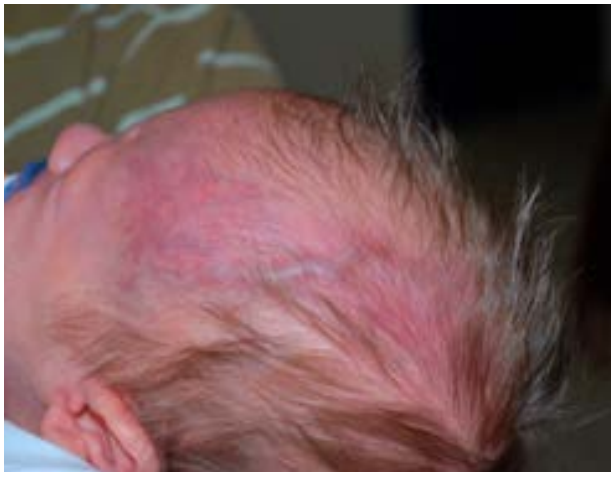


Figure 3: *case 1 showing diffuse reticulate zones of red to purple skin discoloration with underneath prominent veins located on the scalp, jaw and neck.*



being regarded as the most sensitive having a diagnostic rate of 66.7% in one study (2,6).

The current treatment options are inadequate and consist mainly of supportive care, including debulking and orthopedic surgery, amputation, sclerotherapy, and psychological and nutritional support. Relapse of overgrowth following surgery is common and often leads to re-intervention. Multiple pharmacological inhibitors of PI3K, APK, and mTOR are under development (examples are: BYL719, sirolimus, and metformin). These medical therapies, which are currently under evaluation in clinical trials, may suppress the overgrowth, resulting in modest reduction or relative stabilization, but aren't considered to be curative (9-13). A recent study on the efficacy and safety of low dose sirolimus suggests that it can slow down overgrowth but also has a high rate of discontinuation due to significant side effects. A case-by-case approach is so warranted (11).

Follow-up of patients is important since most of the phenotypes can be progressive, as is the case in our patient with Progressive Macrodactyly.

Conclusion

Our two cases are an illustration of the fact that multiple variants in a single gene can result in completely different phenotypes. An absence of a genotype-phenotype correlation has been suggested in recent literature. Our case of Macrodactyly is further supporting this concept since the variant in our patient differs from the variants most commonly described.

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Figure 4: *case 2 at birth showing an enlargement of the first and second left toe*

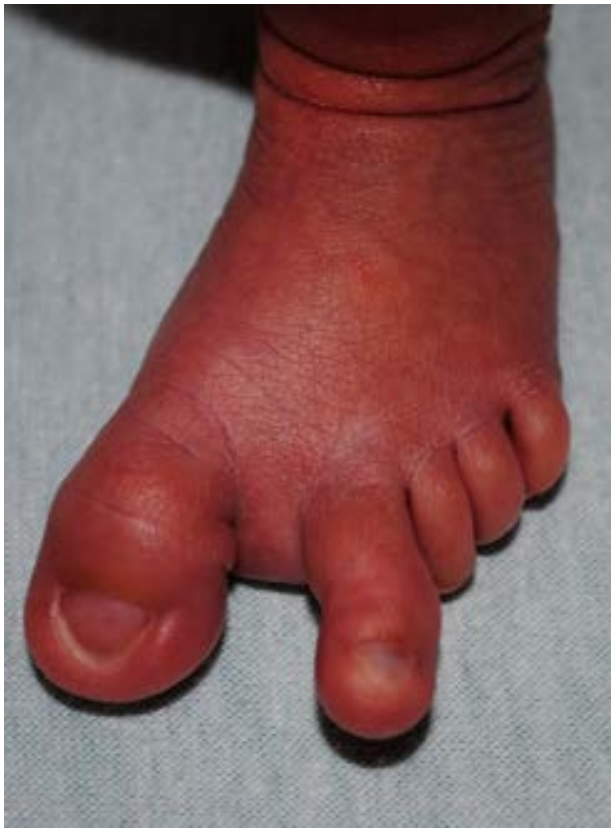

Figure 5: *case 2 at the age of 9 months showing a further enlargement of the involved toes and foot and also of the ipsilateral lower limb.*