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Abstract: Isolated macrodactyly (OMIM 155500) belongs to a heterogeneous group of overgrowth syndromes. It is a congenital anomaly resulting in enlargement of all tissues localized to the terminal portions of a limb and caused by somatic mutations in the phosphatidylinositol 3-kinase catalytic alpha (PIK3CA, OMIM 171834) gene. Here we report a Hungarian girl with macrodactyly and syndactyly. Genetic screening at hotspots in the PIK3CA gene identified a mosaic mutation (c.1624 G>A, p.Glu542Lys) in the affected tissue, but not in the peripheral blood. To date, this somatic mutation has been reported in eight patients affected by different forms of segmental overgrowth syndromes. Detailed analysis of the Hungarian child and previously reported cases suggests high phenotypic diversity associated with the p.Glu542Lys somatic mutation. The identification of the mutation provides a novel therapeutic modality for the affected patients: those who carry somatic mutations in the PIK3CA gene are potential recipients of a novel "repurposing" approach of rapamycin treatment.

Dr. Alain Verloes

Editor in Chief

European Journal of Medical Genetics

Dear Dr. Alain Verloes,

Re: **"Somatic mosaicism of the PIK3CA gene identified in a Hungarian girl with macrodactyly and syndactyly" Tripolszki et al.**

We would be grateful if you would kindly consider this manuscript for publication as a Clinical Research in the European Journal of Medical Genetics.

Here we report a 4-year-old Hungarian girl affected by macrodactyly and syndactyly. The performed genetic screening revealed a somatic mutation of the *PIK3CA* gene (c.1624 G/A, p.Glu542Lys). This mutation has been previously reported in other patients affected by different types of overgrowth syndrome. Here we have reviewed the wide clinical spectrum associated with the same somatic mutation.

The authors state no conflict of interests.

Thank you for your consideration.

Sincerely yours,

Nikoletta Nagy

Corresponding author

Somatic mosaicism of the *PIK3CA* gene identified in a Hungarian girl with macrodactyly and syndactyly

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Running title: Somatic PIK3CA mutation

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Abstract

Isolated macrodactyly (OMIM 155500) belongs to a heterogeneous group of overgrowth syndromes. It is a congenital anomaly resulting in enlargement of all tissues localized to the terminal portions of a limb and caused by somatic mutations in the *phosphatidylinositol 3-kinase catalytic alpha* (*PIK3CA*, OMIM 171834) gene. Here we report a Hungarian girl with macrodactyly and syndactyly. Genetic screening at hotspots in the *PIK3CA* gene identified a mosaic mutation (c.1624G>A, p.Glu542Lys) in the affected tissue, but not in the peripheral blood. To date, this somatic mutation has been reported in eight patients affected by different forms of segmental overgrowth syndromes. Detailed analysis of the Hungarian child and previously reported cases suggests high phenotypic diversity associated with the p.Glu542Lys somatic mutation. The identification of the mutation provides a novel therapeutic modality for the affected patients: those who carry somatic mutations in the *PIK3CA* gene are potential recipients of a novel "repurposing" approach of rapamycin treatment.

Key words

overgrowth syndromes, macrodactyly and syndactyly, *PIK3CA* gene, somatic mutation, phenotypic diversity

Introduction

Macrodactyly (OMIM 155500) refers to a rare congenital malformation occurring in approximately 1 in 100,000 live births and is characterized by an increase in the size of all the structures of the limbs, including soft tissues, bones, vessels, nerves and skin (1). It typically affects the terminal portions of the limb within a "nerve territory" and the individual peripheral nerve is both enlarged and elongated (1). Recently macrodactyly has been added to the growing list of overgrowth syndromes caused by somatic mutations of the *phosphatidylinositol 3-kinase catalytic alpha* (*PIK3CA*, OMIM 171834) gene (1-3).

Further somatic overgrowth diseases associated with mosaic *PIK3CA* mutations include fibroadipose hyperplasia and non-classifiable conditions characterized by muscular, boney and fatty tissue overgrowth, congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal abnormalities (CLOVES) syndrome, hemihyperplasia multiple lipomatosis, and the brain overgrowth conditions megalencephaly capillary malformation and megalencephaly-polymicrogyria-hydrocephalus syndrome (MPPH) (2, 4-8). The phenotypic heterogeneity in these syndromes is attributed to the location of the cells bearing the mutation and to the proportion of the affected cells in the patient's tissues (9).

Here we describe a 4-year-old Hungarian patient with isolated macrodactyly and syndactyly caused by the c.1624G>A, p.Glu542Lys somatic mutation of the *PIK3CA* gene. We also provide detailed comparison of the clinical symptoms of the Hungarian patient with previously reported cases having the same p.Glu542Lys somatic *PIK3CA* mutation.

Clinical report

A 4-year-old Hungarian girl was referred to the Department of Medical Genetics (University of Szeged, Szeged, HUNGARY) with isolated macrodactyly on the third and

fourth fingers of the left hand (Fig. 1a). X-ray imaging proved that the disease is characterized not only by the overgrowth of the soft tissues but also by the overgrowth of the bones of the affected fingers (Fig. 1b). In addition to macrodactyly, syndactyly was associated with concrescence of the fingers and was constrained to the soft tissues of the affected fingers and not to the bones (Fig. 1a,b). The macrodactyly and syndactyly of the left hand was present at birth and slowly progressed with the growth of the child. On examination, no vascular abnormality was present. There was no associated abnormality of the internal organs. Other body parts were symmetric and equally developed. The patient's parents were clinically unaffected, and they were not aware of any other family members with either macrodactyly or syndactyly (Fig. 1c).

The performed genetic investigation was approved by the Internal Review Board of the University of Szeged. The study was conducted according to the Principles of the Declaration of Helsinki. After informed consent was obtained from the parents, peripheral blood sample and deep surgical excision of the affected left hand were taken in order to perform genetic analysis. Genomic DNA was isolated with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Primer sequences were obtained from the UCSC Genome Browser. The coding regions and flanking introns of the *PIK3CA* gene were amplified and sequenced with a traditional capillary sequencer (ABI Prism 7000). Since traditional sequencing did not identify any putative causative variant of the *PIK3CA* gene, mutational hotspots were screened using an in-house PCR-based restriction fragment assay which has been previously described (10) (Cambridge, UK). Examining the region surrounding codon 542, a PCR product of 180 base pairs in length was amplified and digested with the *XbaI* restriction enzyme for 2 hours at 37°C. The resulting fragments were detected by fluorescent read-out using GeneMapper® software with GeneScan LIZ 500 (Life Technologies Corporation) as the size standard. A mosaic mutation at codon 542 (c.1624G>A,

p.Glu542Lys) was identified in the affected tissue with 4% mutation burden (Fig. 2a). This mutation was not present in the genomic DNA sample isolated from the peripheral blood of the patient (Fig. 2b). This genetic analysis confirmed that the development of macrodactyly and syndactyly of the third and fourth fingers of the left hand are the consequence of the mosaicism of the p.Glu542Lys mutation in the *PIK3CA* gene.

Discussion

The case reported in this study presented with macrodactyly and syndactyly of the third and fourth fingers of the left hand. Genetic investigation identified a somatic missense gain-of-function mutation (p.Glu542Lys) of the *PIK3CA* gene (ClinVar database, http://www.ncbi.nlm.nih.gov/clinvar/ accession number: SCV000258982). This mutation affects the helical domain of the p110α catalytic subunit of the PI3K protein (11). Functional studies have previously shown that this p.Glu542Lys variant caused hyperactivation of AKT, a down-stream target of PI3K in the nerve cells of a patient with macrodactyly (1). This particular mutation has been reported in eight patients with different forms of segmental overgrowth (Table 1) (1-3, 12).

Patients with somatic p.Glu542Lys mutation of the *PIK3CA* gene show high phenotypic diversity; Kurek et al. (2012), for example, described a female and a male patient affected by CLOVES syndrome. In addition to macrodactyly, both patients developed lipomatous overgrowth of the trunk and the limbs and vascular anomalies including lymphatic, capillary and venous malformations. The affected female patient also had a hypoplastic right kidney (2). A subsequent study reported the prenatal diagnosis of CLOVES syndrome in a 27-week-old fetus carrying the same somatic mutation of the *PIK3CA* gene. The observed clinical symptoms at birth were asymmetric chest and abdomen, bilateral multicystic malformations and asymmetric growth of the left leg with macrodactyly of the left

foot and a sandal gap between the first and second toes (3). Rios et al. (2013) reported two patients with the same somatic mutation; both were affected by macrodactyly. However, one of the patients was also affected by true muscular hemihypertrophy, which was also attributed to the presence of the somatic p.Glu542Lys *PIK3CA* mutation. A subsequent study has also reported three further patients with facial infiltrating lipomatosis, which was attributed to the presence of the somatic p.Glu542Lys mutation (12). These patients did not exhibit macrodactyly.

The high phenotypic diversity associated with the somatic p.Glu542Lys mutation might be explained by the different time points, in which the mutational events occurred during embryogenesis. Patients with CLOVES syndrome might have developed the same somatic p.Glu542Lys mutation earlier during the embryogenesis than the ones with regional overgrowth (macrodactyly or facial infiltrating lipomatosis).

Genetic analysis has a huge significance for these patients, as once the genetic cause is determined, pharmacological intervention could be considered as a therapeutic option. In the interim, there are no clinically approved therapies for this condition; however there is a theoretical possibility that small molecule inhibitors of the PI3K-AKT-mTOR signaling pathway could be effective therapies for these patients (13, 14). Rapamycin (sirolimus) indirectly targets PI3K and may also be useful in treating macrodactyly (13, 14). In addition, rapamycin has been reported to be effective in isolated cases of allied conditions and may promote a breakthrough in the treatment of macrodactyly and overgrowth syndromes. However, long-term safety data of this treatment in PIK3CA-related overgrowth is currently lacking and, thus, indicates the need for formal clinical trials to evaluate safety and efficacy. In light of the current situation, detailed genetic investigation and publication of these isolated cases is essential.

Acknowledgements

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Conflict of Interest: The authors declare that they have no conflict of interest.

Figure Legends

Figure 1. Macrodactyly and syndactyly in a 4-year-old girl. (a) Clinical features and **(b)** bone radiographs of the patient. **(c)** The patient's family is clinically asymptomatic.

Figure 2. Genetic investigation of the Hungarian patient with macrodactyly and syndactyly. (a) DNA was extracted from the affected left hand and subjected to a PCR restriction assay, which identified the *PIK3CA* p.Glu542Lys mutation at 4% mutation burden. **(b)** In DNA extracted from the peripheral blood, the peak indicating the presence of the mutation was absent in the digested PCR product, indicating the mosaicism of the mutation.

Table 1. Summary of the phenotypic diversity of the patients with somatic p.Glu542Lys PIK3CA mutation.

Patients	Index Patient	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Clinical diagnosis	Macro-dactyly, Syndactyly	Macro-dactyly	Macro-dactyly, Muscular hemi-hypert-rophy	Facial infiltrating lipo-matosis	Facial infiltrating lipo-matosis	Facial infiltrating lipo-matosis	CLOVES syndrome	CLOVES syndrome	CLOVES syndrome
Publication	This report	Rios et al. 2013	Rios et al. 2013	Maclellan et al. 2014	Maclellan et al. 2014	Maclellan et al. 2014	Kurek et al. 2012	Kurek et al. 2012	Emrick et al. 2014
Percentage (%) of somatic mosaicism	4	NR	NR	23	30	16-18	8	6-13	38
Lipomatous overgrowth									
Face	NR	NR	NR	+	+	+	NR	NR	NR
Trunk	NR	NR	NR	NR	NR	NR	+	+	+
Limb(s)	+	+	+	NR	NR	NR	+	+	+
Vascular anomalies									
Lymphatic malformation	NR	NR	NR	NR	NR	NR	+	+	+
Capillary malformation	NR	NR	NR	NR	NR	NR	+	+	+
Venous malformation	NR	NR	NR	NR	NR	NR	+	+	+
Musculoskeletal									
Macrodactyly	+	+	+	NR	NR	NR	+	+	+
Limb asymmetry	NR	NR	NR	NR	NR	NR	+	+	
Scoliosis	NR	NR	NR	NR	NR	NR	+		
True muscular hemihypertrophy	NR	NR	+	NR	NR	NR	NR	NR	NR
Other findings	NR	NR	NR	NR	NR	NR	Hypo-plastic right kidney	NR	Epidermal nevi

NR=not reported

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REPLY TO THE REVIEWERS

Ref. No.: EJMG-D-15-00318

Title: Somatic mosaicism of the PIK3CA gene identified in a Hungarian girl with macrodactyly and syndactyly

Editor's comments:

Dissemination of the information about published genetic and genomic variants is important. As suggested in the Guidelines for Authors, you have to submit DNA variants or CNV mentioned in this article to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) or to another public reference database before your publication could be accepted. Note that many databases (including ClinVar) accept to keep submission private until final acceptance of a manuscript. Please mention the database and quote accession number(s) in the manuscript.

Reply: Thank you for your suggestion, we have submitted our data to the ClinVar database (accession number: SCV000258982).

Reviewers' comments:

Reviewer #1:

The authors presented a patient with macrodactyly and syndactyly and detected a mosaic somatic mutation of the PIK3CA gene (p.Glu542Lys) in the affected tissue. They have compared the phenotypic data of their patient with other patients with different forms of segmental overgrowth that also carry somatic PIK3CA p.Glu542Lys mutation.

This is a concise and well written case report that presents macrodactyly combined with syndactyly as a new feature of somatic PIK3CA p.Glu542Lys mutation. Also, comparison of

clinical features of patients with the somatic PIK3CA p.Glu542Lys mutations is very helpful in understanding the broad aspects of phenotypic variability of this mutation. I have the following minor comments:

Comment 1:

In the table comparing the patients with PIK3CA p.Glu542Lys mutation please add a column related to the percentage of somatic mosaicism of this mutation in the affected organs.

Reply 1: Thank you for your comment, a new column highlighting the percentage of somatic mosaicism in the affected organs have been added to the revised version of Table I.

Comment 2:

Please indicate the type of PIK3CA p.Glu542Lys mutation as gain-of-function mutation in the text.

Reply2: The p.Glu542Lys mutation of the PIK3CA gene has been indicated as gain-of-function mutation in the Discussion part of the revised version of the manuscript.

Comment 3:

Please fill.

Reply3: The blank areas have been filled with NR (not reported) in the revised version of Table I.

Reviewer #3:

The manuscript is a case description of a patient with isolated macrodactyly and mosaicism for a previously described mutation in PIK3CA. The authors present a summary of previously reported patients with the same mutation and give an outlook on future therapeutic options.

The report is generally well written and interesting. As a minor comment, I suggest to change the nomenclature of the mutation according to HGVS. On page 6, line 7, I would prefer a different sentence structure: "… different time points of the mutational events during embryogenesis."

Reply: Thank you very much for your comments. The nomenclature of the mutation has been corrected according to the recommendation of HGVS. On page 6, line 7, the structure of the sentence has been changed from „… different time points of the mutational events during embryogenesis." to „… different time points, in which the mutational events occurred during embryogenesis."

Figure 1

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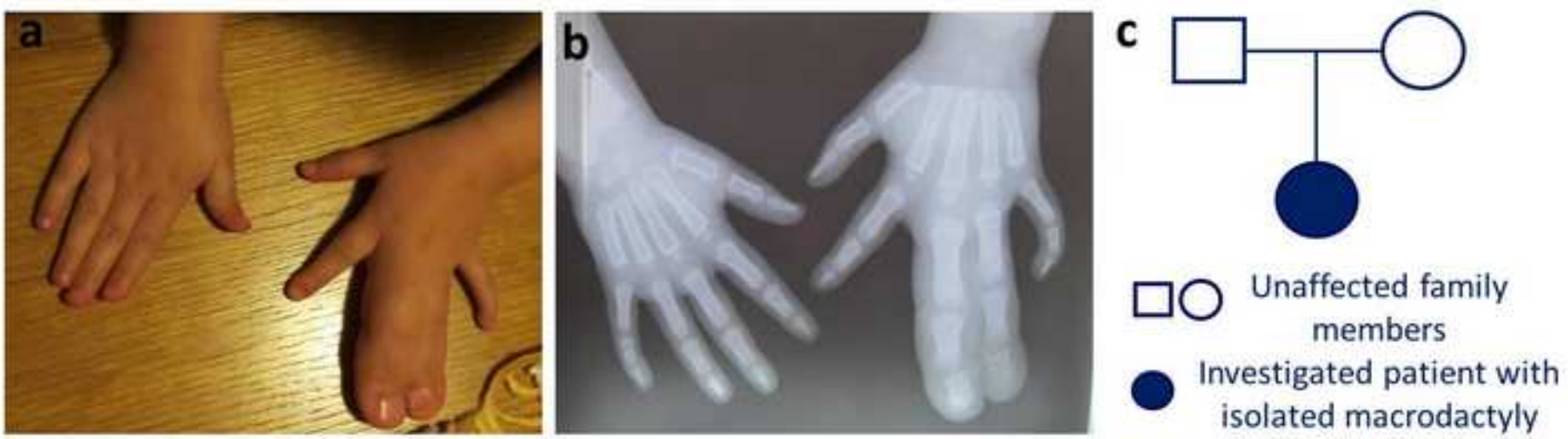

Figure 2

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