

# Phenotypic and genetic spectrum of isolated macrodactyly: somatic mosaicism of PIK3CA and AKT1 oncogenic variants

**Wen Tian**

Beijing Jishuitan Hospital

**Yingzhao Huang**

Peking Union Medical College Hospital

**Liyang Sun**

Beijing Jishuitan Hospital

**Yang Guo**

Beijing Jishuitan Hospital

**Sen Zhao**

Peking Union Medical College Hospital

**Mao Lin**

Peking Union Medical College Hospital

**Xiying Dong**

Peking Union Medical College Hospital

**Wenyao Zhong**

Beijing Jishuitan Hospital

**Yuehan Yin**

Beijing Jishuitan Hospital

**Zefu Chen**

Peking Union Medical College Hospital

**Nan Zhang**

Beijing Jishuitan Hospital

**Yuanqiang Zhang**

Peking Union Medical College Hospital

**Lianlei Wang**

Peking Union Medical College Hospital

**Jiachen Lin**

Peking Union Medical College Hospital

**Zihui Yan**

Peking Union Medical College Hospital

**Xin Zhuang Yang**

Peking Union Medical College Hospital

**Junhui Zhao**

Beijing Jishuitan Hospital

**Guixing Qiu**

Peking Union Medical College Hospital

**Jianguo Zhang**

Peking Union Medical College Hospital

**Zhihong Wu**

Peking Union Medical College Hospital

**Nan Wu** (✉ [dr.wunan@pumch.cn](mailto:dr.wunan@pumch.cn))

Peking Union Medical College Hospital

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## Research

**Keywords:** Macroductyly, Phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA), AKT1 serine/threonine kinase 1 (AKT1), Somatic mosaicism, Proteus syndrome

**Posted Date:** January 1st, 2020

**DOI:** <https://doi.org/10.21203/rs.2.19798/v1>

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**Version of Record:** A version of this preprint was published on October 14th, 2020. See the published version at <https://doi.org/10.1186/s13023-020-01572-9>.

# Abstract

**Background:** Isolated macrodactyly is a severe congenital hand anomaly with functional and physiological impact. Known causative genes include *PIK3CA*, *AKT1* and PTEN. The aim of this study is to gain insights into the genetics basis of isolated macrodactyly.

**Results:** We enrolled 24 patients with isolated macrodactyly. Four of them were diagnosed with Proteus syndrome based on skin presentations characteristic to this disease. Targeted next-generation sequencing was performed using patients' blood and affected tissues. Overall, 20 patients carry mosaic *PIK3CA* pathogenic variants, i.e. p.His1047Arg (N=7), p.Glu542Lys (N=6), p.Glu545Lys (N=2), p.His1047Leu (N=2), p.Glu453Lys (N=1), p.Gln546Lys (N=1) and p.His1047Tyr (N=1). Four patients who met the diagnostic criteria of Proteus syndrome carry mosaic *AKT1* p.Glu17Lys variant. Variant allele frequencies of these mosaic variants obtained through next-generation sequencing range from 10% to 33%. In genotype-phenotype correlation analysis of patients with *PIK3CA* variant, we found that patients with the macrodactyly of the upper and lower limbs tend to carry *PIK3CA* variants located in the kinase and helical domain ( $P=0.011$ ).

**Conclusions:** Mosaic *PIK3CA* and *AKT1* variants can be found in all of our samples with isolated macrodactyly. Insights into phenotypic and genetic spectrum of isolated macrodactyly may be helpful in perusing a more precise and effective management of isolated macrodactyly.

## 1 Introduction

Macrodactyly is a rare congenital anomaly characterized by the overgrowth of all histological component of the digits. The incidence of macrodactyly is approximately 1/50000 to 1/100000 live births, which varies with regional and ethnical demographics [1, 2]. Dismorphic appearances could potentially lead to psychological and social problems. Due to its highly variable phenotypic appearances and low frequency of occurrence, macrodactyly can be a great challenge for hand surgeons, and precisely for its rarity, its operation still lacks a standard guideline [3]. Surgical procedures such as soft tissue debulking, physal arrest and amputation are able to produce acceptable functional and cosmetic outcomes, but improvements and modifications are still needed (5).

Somatic mosaicism in Phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) has been identified as the cause of various overgrowth disorders including isolated macrodactyly, congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal/scoliosis/spinal abnormalities (CLOVE syndrome), Kippel-Trenaunay syndrome (KTS) and megalencephaly-capillary malformation (MCAP) and dysplastic megalencephaly (DMEG). Although these disorders differ from each other, tissue enlargement in parts of the body is a distinct feature common to all. *PIK3CA* is a proto-oncogene, encoding the alpha catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase, a member of the phosphatidylinositol 3-kinase (PI3K) enzyme family [4]. *PIK3CA* is one of the most frequently mutated genes in human tumors [4]. PI3K-AKT1-mTOR signaling mediates cell proliferation, survival and metabolism, activating of this

pathway is involved in tumorigenesis [5]. Mutated PIK3CA protein promotes cell growth and survival by enhancing lipid kinase activity and constitutively activating AKT1 and mTOR signaling.

Although somatic mosaicism of PIK3CA and AKT1 variants in syndromes involving macrodactyly has been well characterized [6], its mutational architecture and relationship between genotypes and phenotypes in isolated macrodactyly is yet to be elucidated, and the identification of low-level mosaicism in affected tissue is rather challenging. There are few studies that employ the Next generation sequencing (NGS)-based method to explore the genetic basis of isolated macrodactyly. In this study, we identified mosaic PIK3CA and AKT1 variants in a cohort of 24 isolated macrodactyly patients by an NGS-based method. We further assessed the genotype-phenotype correlation caused by PIK3CA variants in isolated macrodactyly.

## 2 Results

### Clinical characteristics of the subjects

Clinical and molecular characteristics of these 24 subjects are described in Table 1. Representative photographs of nine isolated macrodactyly patients are shown in Fig. 1. Twenty-two of 24 patients displayed asymmetric and disproportionate overgrowth in their hands or feet at birth. The remaining two patients had a later onset between six and twelve months after birth. Our sample ranged in age from 1 to 34 years, though except one 34-year-old patient, most are less than 15 years of age, with a median age of 5. A slight male predominance was observed (16 males versus eight females, binomial  $P = 0.152$ ) in our cohort. Fourteen patients had exclusive involvement in the lower extremities, and nine had macrodactyly exclusively in the upper extremities, suggesting a slight predominance of the lower extremities (binomial  $P = 0.405$ ). Only one patient had overgrowth of both the upper and the lower extremities. Twenty of 24 patients had unilateral involvement, including 12 V.S. 8 patients with right V.S. left side affected (binomial  $P = 0.503$ ). Four of 24 patients had bilateral involvements. The number of affected digits ranged from one to seven, with an average of 2.7. The second digit is the most frequently affected digit ( $N = 22$ ), followed by the third ( $N = 17$ ). More than half of our patients (14/24) had two affected digits, and the combination of their respective locations at the second- and third-digit overgrowth ( $N = 10$ ) was more frequently observed than double-digits enlargement of the first and the second ( $N = 4$ ; binomial  $P = 0.180$ ). In addition, no other combinations were observed when patients have two affected digits. Five of 24 patients had syndactyly, and all of them were presented with syndactyly of 2–3 toes.

Table 1  
Summary of the clinical and molecular findings of studied subjects.

Patient No.	Gender	Age	Syndactyly	Affected limb	Affected digits	Gene	Variant	VAF
1	M	11	N	R-H	2,3	AKT1	c.49G > A (p.Glu17Lys)	22.03%
2	M	11	N	L-F	1,2,3,4,5	AKT1	c.49G > A (p.Glu17Lys)	11.16%
3	M	2	N	R-H	2,3	AKT1	c.49G > A (p.Glu17Lys)	9.93%
4	F	10	N	L-H	3,4,5	AKT1	c.49G > A (p.Glu17Lys)	20.57%
5	F	6	N	B-F, R-H	L-F: 2,3; R-F:1,2; R-H:2,3	PIK3CA	c.1357G > A (p.Glu453Lys)	11.10%
6	M	3	N	R-F	2	PIK3CA	c.1624G > A (p.Glu542Lys)	24.48%
7	M	4	Y	L-F	2,3	PIK3CA	c.1624G > A (p.Glu542Lys)	17.15%
8	M	5	Y	L-F	1,2,3,4	PIK3CA	c.1624G > A (p.Glu542Lys)	20.95%
9	M	4	N	L-F	2,3	PIK3CA	c.1624G > A (p.Glu542Lys)	17.10%
10	F	4	N	R-F	2,3	PIK3CA	c.1624G > A (p.Glu542Lys)	27.58%
11	M	3	N	R-F	1,2	PIK3CA	c.1624G > A (p.Glu542Lys)	17.79%
12	M	2	N	L-F	1,2,3	PIK3CA	c.1633G > A (p.Glu545Lys)	19.11%
13	M	2	Y	R-F	2,3	PIK3CA	c.1633G > A (p.Glu545Lys)	27.31%
14	M	11	N	B-F	L: 1,2,3; R:1,2,3,4	PIK3CA	c.1636C > A (p.Gln546Lys)	24.50%
15	F	13	N	B-F	2	PIK3CA	c.3139C > T (p.His1047Tyr)	18.94%
16	M	1	N	R-H	2,3	PIK3CA	c.3140A > G (p.His1047Arg)	25.63%

M: male; F: female; Y: Yes or present; N: Not present; L: left; R: right; B: bilateral; VAF: variant allele frequency. NA: Not available.

Patient No.	Gender	Age	Syndactyly	Affected limb	Affected digits	Gene	Variant	VAF
17	M	2	Y	R-F	1,2	PIK3CA	c.3140A > G (p.His1047Arg)	23.29%
18	F	5	Y	L-F	2,3	PIK3CA	c.3140A > G (p.His1047Arg)	21.45%
19	M	2	N	R-H	1,2	PIK3CA	c.3140A > G (p.His1047Arg)	25.57%
20	F	10	N	R-H	1,2,3,4,5	PIK3CA	c.3140A > G (p.His1047Arg)	10.36%
21	M	2	N	L-F	2,3	PIK3CA	c.3140A > G (p.His1047Arg)	20.03%
22	F	6	N	B-H	L:5; R:1,2,3	PIK3CA	c.3140A > G (p.His1047Arg)	15.82%
23	F	3	N	R-H	5	PIK3CA	c.3140A > T (p.His1047Leu)	33.38%
24	M	34	N	R-H	1,2	PIK3CA	c.3140A > T (p.His1047Leu)	18.99%
M: male; F: female; Y: Yes or present; N: Not present; L: left; R: right; B: bilateral; VAF: variant allele frequency. NA: Not available.								

Patients 1 to 4 had variable presentations of cerebriform connective tissue nevi adjacent to overgrown digits. Therefore, diagnoses of Proteus syndrome were established in these patients.

#### Genetic characteristics

After genetic testing and interpretation, molecular diagnoses were achieved in all of our patients. In all four patients who met the diagnostic criteria of Proteus syndrome, we identified an AKT1 c.49G > A (p.Glu17Lys) variant, which is the only known variant leading to Proteus syndrome [10]. Variant allele frequencies (VAFs) of AKT1 in affected tissues range from 10–22%, with an average variant frequency of 16%. No variant read was identified in blood DNA (Table 1).

In 20 patients with isolated macrodactyly who do not meet the diagnostic criteria of Proteus syndrome, we identified and confirmed pathogenic variants in PIK3CA from 20 patients (Table 1). The most commonly observed variants are PIK3CA p.His1047Arg (N = 7), followed by p.Glu542Lys (N = 6), p.Glu545Lys (N = 2), p.His1047Leu (N = 2), p.Glu453Lys (N = 1), p.Gln546Lys (N = 1) and p.His1047Tyr (N = 1) variations (Table 1). VAFs in affected tissues identified in range from 10–33% with the average of 21% (Table 1). None of these variants was identified in peripheral blood samples.

All of the seven variants have been previously reported to cause developmental disorders [5, 6, 11, 12]. These variants were either predicted or validated to have a gain-of-function mechanism. In vivo studies

demonstrated that PIK3CA congenic variants could induce oncogenic transformation in chicken embryo fibroblasts by enhancing lipid kinase activity and activating mTOR and AKT1 signaling [13]. All seven variants were absent from the Deciphering Disorders Involving Scoliosis and COmorbidities (DISCO, <http://discostudy.org/>) study composed of 4000 exome sequencing data of Chinese population [7–9]. PIK3CA p.Glu453Lys, p.Glu542Lys, p.Gln546Lys and p.His1047Tyr were absent from the Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org>). PIK3CA p.Glu545Lys, p.His1047Leu, p.His1047Arg and AKT1 p.Glu17Lys were present at extremely low frequencies in gnomAD, with an allele frequency of 4e-6. Though the PIK3CA p.Gln546Lys, p.His1047Tyr and p.Glu453Lys variant had been observed in other forms of PROS [6, 11, 14–16], this is the first time for them to be identified in isolated macrodactyly.

PIK3CA protein has five functional domains, i.e. PI3K-ABD, PI3K-RBD, C2 PI3K-type, PIK helical and PI3K/PI4K kinase domain. p.Glu453Lys variant was located in the C2 domain. p.Glu542Lys, p.Glu545Lys and p.Gln546Lys variants occurred in adjacent amino acids of the helical domain. p.His1047Arg, p.His1047Tyr and p.His1047Leu variants were located at the kinase domain of PIK3CA (Fig. 2). The majority of established functional variants of PIK3CA cluster were found in the kinase and helical domains [17], which is in consistency with our finding in this study. In the 20 patients carrying pathogenic PIK3CA variants, nine had variants in the helical domain and ten were located in the kinase domain, and the remaining one variant located in the C2 domain.

#### Genotype-phenotype correlation

We then analyzed the potential correlation between subjects' phenotypes (i.e. macrodactyly of the upper or the lower limb, the number of affected digits, with or without syndactyly) and the protein location of variants in patients carrying the PIK3CA variant (Table 2).

Table 2  
Genotype-phenotype correlation

	In helical domain	In kinase domain
Affected limb		
Upper limb	0	6
Lower limb	9	4
P-Value	0.011	
Affected digits		
≤ 3	5	8
≥ 3	4	2
P-Value	0.350	

All six (100%) patients presented with upper limb overgrowth carried variants in the kinase domain. In contrast, only four of thirteen (31%) patients who had overgrowth in the lower limbs had their variants located in the kinase domain. Therefore, there is a strong evidence for an association between the location of a genetic variant and the locus of macrodactyly, i.e. in the upper or lower limbs ( $P = 0.011$ ; determined by Fisher's exact test). We also compared the VAFs between the two phenotypically different groups, but no significant correlation was observed.

Thirteen patients have one to two affected digits, and eight of them carried variants located in the kinase domain. Six patients have more than two affected digits, and two of them had a variant in the kinase domain. Though it seemed that in patients with less than three affected digits, variants were more likely to be located in the kinase domain, and vice versa, this observation did not prove to be statistically significant ( $P = 0.350$ ; determined by Fisher's exact test).

### 3 Discussion

In this study, all of macrodactyly patients in our report whose DNA samples underwent a targeted NGS-based sequencing were identified with either a pathogenic PIK3CA or AKT1 mosaic variant. No pathogenic or likely pathogenic variant was detected in other genes that are currently known to be associated with macrodactyly, suggesting that somatic mosaicism in PIK3CA and AKT1 is a predominant cause of isolated macrodactyly. This inference is supported by a recent study in which nine of twelve subjects with non-syndromic macrodactyly who underwent targeted Sanger sequencing were positive for somatic mosaicism in PIK3CA [14].

Previous studies mainly applied the Sanger sequencing method in mosaic variant detection [10, 18], which is not sensitive enough to detect low-level mosaicism [18], for that the mosaicisms in our sample patients no. 2, 3, 5, 8 and 20 would hardly be recognized by Sanger sequencing (Supplementary material). In this report, we demonstrated that an NGS-based method could detect a mosaicism even when it was as low as 10%. Considering the limitation of Sanger sequencing in identifying mosaic variants and the wide spectrum of pathogenic variants (eight distinct variants identified in this study) [19], we employed the NGS-based deep sequencing method as our first-line in molecular diagnosis of isolated macrodactyly.

In consistency of our report, most commonly observed PIK3CA somatic variants in PIK3CA-related overgrowth syndrome (PROS) were p.His1047Arg, p.His1047Leu, p.Glu545Lys and p.Glu542Lys [11]. Information on the level of mosaicism can be acquired through Sanger sequencing and NGS [11, 20], and remarkable difference in mosaic level of the different samples collected from the same subject was observed [11]. Seven variants in PIK3CA and one variant in AKT1 identified in this study have previously been described [11, 21]. However, we observed a significant heterogeneity in phenotypic presentations even for the same variant, which might be a consequence of the different times at which variants were acquired [22]. The exact site of variant-induced affection in the human body might also have an impact on the phenotypic presentations. It has been observed that PROS not involving the brain are usually caused by PIK3CA cancer hot-spot variants, e.g. p.Glu542Lys, p.His1047Arg and p.Glu453Lys [6, 11], while PROS involving the brain are usually caused by rare variants. This phenomenon is also described in our report.

Furthermore, we showed that macrodactyly in the upper limb are mostly caused by variants in the kinase domain, whereas PROS involving the lower limb are often a result of variants in the helical domain, and this association shows a robust statistical significance.

We found that VAFs of PIK3CA and AKT1 in affected tissues were around 20%, and no variant was identified in blood samples, further supporting previous findings which suggests that PIK3CA variants are generally not detectable in the blood of patients with Proteus syndrome or with PROS, excluding MCAP [10, 18, 23].

To date, surgical interventions such as simple debulking of the affected tissues or amputation remain the only clinically available options in treating patients with isolated macrodactyly. The detailed procedures and outcome of each surgery are highly variable due to the heterogeneity of patients' clinical presentations. In general, patients are satisfied with their post-surgical appearance [3], but no surgery is capable of fully restoring a digit of its normal function and appearance. Emerging targeted therapy appears to be a hopeful alternative for patients as the genetic pathogenesis of isolated macrodactyly has been largely uncovered. Over the past 25 years, intensive investigations into the genetic basis of tumorigenesis give birth to many revolutionary targeted agents in cancer therapy [24]. Oncogenic PIK3CA and AKT1 variants are crucial in both tumorigenesis and pathogenesis of mosaic overgrowth disorders. Thus, targeted agents previously used in cancer therapy can also be applied in managing patients with mosaic overgrowth disorders associated with PI3K-AKT1-mTOR. Several studies have begun to assess the effects of introducing PI3K-AKT1-mTOR inhibitors in treating PROS patients. The outcome seems promising, regardless of the variant profile of PIK3CA [25, 26]. In these studies, PROS patients administered with PIK3CA or mTOR inhibitors displayed different levels of clinical improvement and a low rate of side effects in short period follow-ups [25, 26]. Still, more clinical trials are pending in evaluating and improving this therapeutic strategy.

In conclusion, our findings demonstrated that isolated macrodactyly is mostly caused by mosaic variants in PIK3CA and AKT1. Patients with the macrodactyly deformities of either the upper or the lower limbs tend to carry variants that were located in the kinase or the helical domain, respectively. With the advent of targeted therapy against PI3K-AKT1-mTOR pathway, a more detailed knowledge in the pathogenesis of isolated macrodactyly will be beneficial for its effective management.

## 4 Conclusions

This study reports the largest series of patients so far with PIK3CA /AKT1-associated macrodactyly, which includes 24 patients with isolated macrodactyly and all of them carried mosaic PIK3CA or AKT1 variants. This study expands the mutational architecture and identified genotype-phenotype correlations, which provides insight into the genetic etiology of mosaic overgrowth syndromes and possibly promotes precise management.

## 5 Methods

Patient selection

This study has a sample of 24 subjects, clinical presented with isolated macrodactyly, who were admitted to Jishuitan Hospital during 2018. Patients whose overgrowth was not limited to the limbs failed to meet the criteria for our sample. Four subjects met the diagnostic criteria of Proteus syndrome. The limb anomalies of these patients were evaluated through physical examinations and X-ray by experienced hand surgeons (WT, LS and YG).

#### Tissue sampling and preparation

Abnormal adipose, skin and nerve tissues were collected during surgery. Genomic DNA was extracted from blood and collected tissues using the Dneasy Blood & Tissue Kit (QIAGEN, Germany) according to the manufacturer's protocol.

#### Genetic test and variant interpretation

A deep targeted NGS was performed on DNA extracted from blood and surgically-removed abnormal tissues of all 24 subjects. Illumina paired-end libraries were prepared from DNA samples and were subjected to targeted capture following a sequencing on the Illumina HiSeq 4000 platform. The mean coverage was 1000 reads with more than 98% regions exceeds 100 reads. In-house developed Peking Union Medical College Hospital Pipeline (PUMP) and variant interpretation were performed following the previously described methods [7–9].

All variants presumed to be pathogenic were subjected to Sanger sequencing. Variant-encoding amplicons were amplified by PCR from genomic DNA obtained from subjects, purified using an Axygen AP-GX-50 kit (lot no. 05915KE1) and sequenced by Sanger sequencing on an ABI3730XL instrument.

#### Genotype-phenotype correlation analysis

Patients carrying variants in the helical and kinase domain of PIK3CA gene were selected to analyze potential genotype-phenotype correlation. We divided the patients into two groups based on the respective domain of an individual's variant. We then compared the location of affected sites (at the upper or lower limbs) and the number of affected digits between the two groups.

#### Genotype-phenotype correlation analysis

SPSS Statistics V15.0 software was used for statistical analyses, and a p-value less than 0.05 was considered statistically significant. Genotype-phenotype correlation was assessed by Fisher's exact test and Student's t test.

## Abbreviations

PIK3CA

Phosphatidylinositol 3-kinase catalytic subunit alpha,

AKT1

AKT serine/threonine kinase 1

PI3K  
Phosphatidylinositol 3-kinase  
NGS  
Next generation sequencing  
VAFs  
Variant allele frequencies  
PROS  
PIK3CA-related overgrowth syndrome  
MCAP  
Megalencephaly-capillary malformation

## Declarations

### 1.1 Ethics approval and consent to participate

Informed consent was acquired during this study. This study is approved by the ethnic committee of Peking Union Medical College Hospital

### 1.2 Consent for publication

The consent for publication was acquired from patients or patients' parents.

### 1.3 Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### 1.4 Competing interests

The authors declare that they have no competing interests.

### 1.5 Funding

This research was funded in part by the The National Key Research and Development Program of China (2016YFC0901500 and 2018YFC0910506), Center for Rare Diseases Research, Chinese Academy of Medical Sciences, Beijing, China (2016ZX310174-4), Beijing JST Research Funding( ZR-201907 and 2019-YJ03), Beijing Jishuitan Hospital Nova Program (XKXX201818), National Natural Science Foundation of China (81822030, 81772299, 81930068, 81772301, 81972132, 81672123 and 81972037), Beijing Natural Science Foundation (7172175 and 7191007) and the Tsinghua University-Peking Union Medical College Hospital Initiative Scientific Research Program, CAMS Initiative Fund for Medical Sciences (2016-I2M-3-003, 2016-I2M-2-006 and 2017-I2M-2-001).

### 1.6 Author Contributions

NW, ZW, GQ, Jianguo Z and Junhui Z conceived and designed the study. WT, LS, GY, ML, YY, NZ, WZ and YH enrolled the cohort. JL, ZC, LW, YZ and YH conducted the experiments. WT, YH, LS, SZ and WN analyzed the data. ZY, XD and ZC conducted the bioinformatic analyses. XY, Jianguo Z, GQ and ZW assisted with study organization and manuscript revision. ZW and Jianguo Z assisted with data interpretation. WT, YH, LS, YG, SZ, XD, XG and NW wrote the manuscript.

## 1.7 Acknowledgments

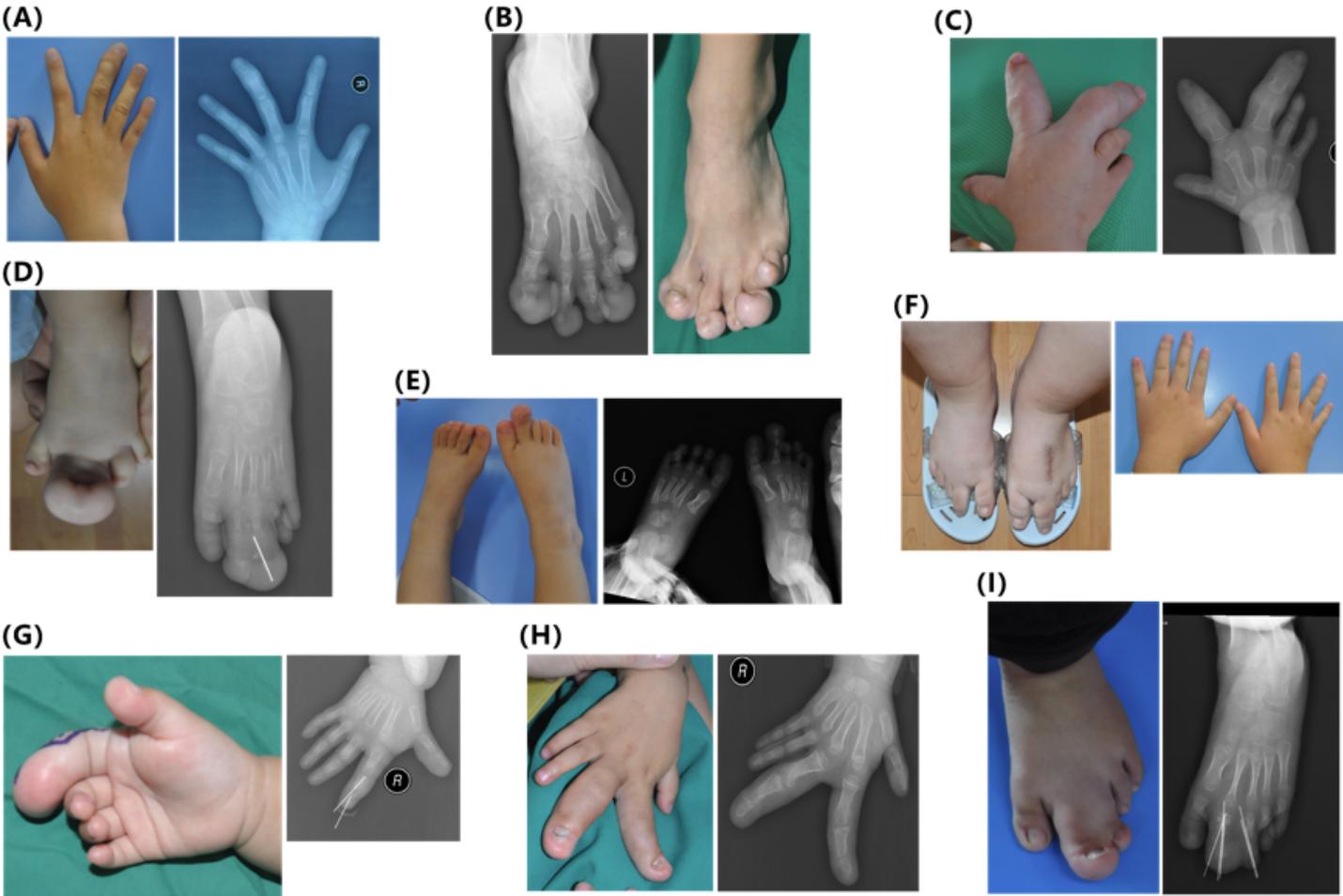
We appreciate all of the patients, their families and clinical staffs.

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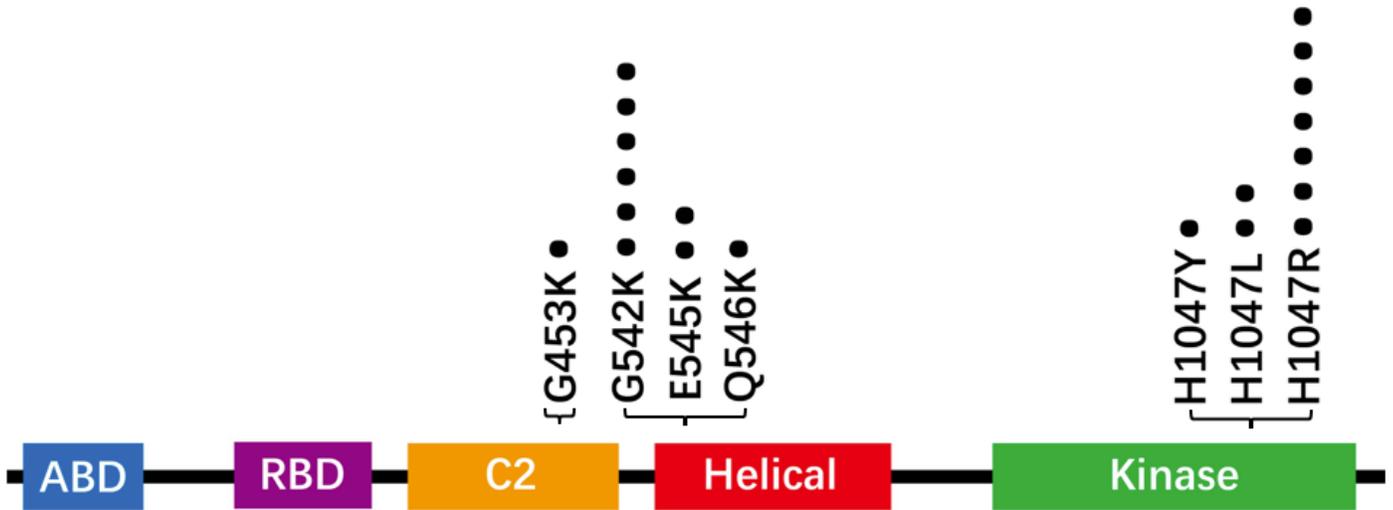
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# Figures



**Figure 1**

Representative clinical photographs of nine macrodactyly patients. (A) Patient No. 1. (B) Patient No. 2. (C) Patient No.3. (D) Patient No.18. (E) Patient No. 6. (F) Patient No. 15. (G) Patient No. 19. (H) Patient No. 16. (I) Patient No. 21.



**Figure 2**

Distribution of PIK3CA variants identified in this study by functional domains. ABD: PI3K-ABD domain. RBD: PI3K-RBD domain. C2: C2 PI3K-type domain. Helical: PIK helical domain. Kinase: PI3K/PI4K kinase domain. A dot represents a PIK3CA variant identified in one patient

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