Clinical Delineation and Natural History of the PIK3CA-Related Overgrowth Spectrum**

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Somatic mutations in the phosphatidylinositol/akt/mTOR pathway cause segmental overgrowth disorders. Diagnostic descriptors associated with PIK3CA mutations include fibroadipose overgrowth (FAO), Hemihiperplasia multiple Lipomatosis (HHML), Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, Scoliosis/skeletal and spinal (CLOVES) syndrome, macrodactyly, and the megalencephaly-capillary malformation (MCAP) syndrome. We set out to refine the understanding of the clinical spectrum and natural history of these phenotypes, and now describe 35 patients with segmental overgrowth and somatic PIK3CA mutations. The phenotypic data show that these previously described disease entities have considerable overlap, and represent a spectrum. While this spectrum overlaps with Proteus syndrome (sporadic, mosaic, and progressive) it can be distinguished by the absence of cerebrofrin connective tissue nevi and a distinct natural history. Vascular malformations were found in 15/35 (43%) and epidermal nevi in 4/35 (11%) patients, lower than in natural history. Vascular malformations were found in 15/35 (43%) and epidermal nevi in 4/35 (11%) patients, lower than in Proteus syndrome. Unlike Proteus syndrome, 31/35 (89%) patients with PIK3CA mutations had congenital overgrowth, and in 35/35 patients this was asymmetric and disproportionate. Overgrowth was mild with little postnatal progression in most, while in others it was severe and progressive requiring multiple surgeries. Novel findings include: adipose dysregulation present in all patients, unilateral overgrowth that is predominantly left-sided, overgrowth that affects the lower extremities more than the upper extremities and progresses in a distal to proximal pattern, and in the most severely affected patients is associated with marked paucity of adipose tissue in unaffected areas. While the current data are consistent with some genotype–phenotype correlation, this cannot yet be confirmed.

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Key words: somatic mosaicism; PIK3CA gene; fibroadipose overgrowth; segmental overgrowth; macrodactyly; CLOVES syndrome

INTRODUCTION

Next generation sequencing has resulted in major advances in understanding the molecular etiology of somatic overgrowth syndromes [Lindhurst et al., 2011; Lindhurst et al., 2012; Kurek et al., 2012; Lee et al., 2012; Raffan and Semple, 2011; Rivière et al., 2012; Shirley et al., 2013]. Since the finding in 2011 that Proteus syndrome is caused by somatic activating mutations in the growth-promoting serine/threonine kinase AKT1, multiple subsequent reports have described activating mutations in other signaling proteins in the same RTK/PI3K/AKT/mTOR growth pathway in several different segmental overgrowth syndromes. The mutated genes include PIK3CA, PIK3R2, AKT3 and mTOR [Lindhurst et al., 2012; Kurek et al., 2012; Lee et al., 2012; Poduri et al., 2012; Rivière et al., 2012; Rios et al., 2013]. Some mutations have been found in more than one phenotypically distinct disorder, and this overlap raises the important question of the relative contributions of underlying genotype, of timing of the mutation and of the precise cell of origin of the founder mutation during development to the ultimate clinical phenotype. A particularly encouraging aspect of recent genetic findings is that the pharmaceutical industry is engaged in a major effort to develop drugs targeting this pathway for use in cancer. This means that these genetic discoveries have brought the prospect of targeted drug trials in segmental overgrowth dramatically closer. Critical to the planning of effective trials will be an understanding of the natural history of the different RTK/PI3K/AKT/mTOR-related overgrowth disorders.

One of the recently described segmental overgrowth phenotypes caused by mutations in the PIK3CA gene is fibroadipose overgrowth (FAO) [Lindhurst et al., 2012]. The major manifestation of this disorder is segmental and progressive overgrowth of subcutaneous, muscular, and visceral fibroadipose tissue, sometimes associated with skeletal overgrowth. We now provide further details of eight of the patients with this disorder previously described in an abbreviated form [Lindhurst et al., 2012] (a ninth patient from that report was not included because she was the subject of a recent clinical report [Tziotzios et al., 2011]) and present 27 additional patients with a broader range of phenotypic manifestations who
RESULTS

The molecular diagnoses in these 35 patients were determined by the presence of a somatic PIK3CA mutation, found in the affected tissues at varying levels, and not in the blood or saliva, as described in each clinical summary and in Table I. We reviewed the clinical and radiological findings in each patient focusing on features described in Lindhurst et al. [2012] and features associated with Proteus syndrome, which is summarized in Table II. The ordering of patients in the table was informally ranked from mild to severe. All patients had overgrowth consistent with what we have previously described as fibroadipose tissue. The assessment of severity was subjective; unilateral was considered milder than bilateral in most cases, a greater number of findings was considered more severe than fewer findings, and more surgeries performed in the past was considered more severe. We did not construct a formal global severity score as this was challenging considering the range of the effects in the patients. Most of the severely affected patients were previously designated as having CLOVES syndrome [Sapp et al., 2007] and had most or all of the characteristic features of CLOVES syndrome.

The most common mutation was the p.His1047Arg (H1047R) occurring in 19/35 (54%) patients. The distribution of the other mutations was: p.His1047Leu (H1047L) in 8/35 (23%) patients, p.Glu542Lys (E545K) in 4/35 (11%) patients, p.Glu542Lys (E542K) in 3/35 (8%) patients, and p.Cys420Arg (C420R) in 1/35 (3%) patients.

There was a qualitative correlation of these genotypes with the overall phenotypes. Twenty-five of 35 (71%) patients had a phenotype most consistent with either FAO, Hemihyperplasia-Multiple Lipomatosis (HHML) or macrodactyly phenotypes, and all but two of these patients had either p.His1047Arg or p.His1047Leu. The other mutations that were found in this group were PIK3CA p.Glu542Lys, and p.Glu545Lys. In those 9/35 (26%) individuals with the CLOVES syndrome phenotype the distribution of mutations was as follows: three with p.His1047Arg, three with p.Glu545Lys, two with p.Glu542Lys, and one with p.Cys420Arg (Fig. 1).

Thirty-one of 35 patients had congenital manifestations, except for one identified between the ages of 2 and 3 months, two between 12 and 18 months, and one at puberty. Four patients had findings that were detectable prenatally. The age range at our evaluation was 1–49 years with the mean age 14.5 years, and the median 7 years. Five patients were greater than 40 years old. The male to female ratio was 1:1.3 (15 males to 20 females, binomial $P \approx 0.5$).

All 35 individuals had asymmetric, disproportionate overgrowth, which was sporadic, and had a progressive course. This overgrowth was predominantly in the limbs or fingers/toes (Fig. 2 A–G). More individuals had involvement of the lower extremities (24/35) than upper extremities (6/35, $P = 0.041$), and three of 35 had overgrowth of both upper and lower extremities. The remaining two of 35 patients had overgrowth involving the midline (back, trunk) and no extremities. The extent of involvement of the extremities in descending order of frequency, was: combinations of toes, feet, leg ($N = 14$) > toes and feet ($N = 6$) > toes only ($N = 3$) > fingers and hands ($N = 2$) or hand and arm ($N = 2$) or toes/feet/leg and fingers ($N = 2$) > legs only ($N = 1$). Two of the patients with the combination of toes, feet and legs affected also had
| Patient | Anatomical source | Tissue mutation level (%) | Cultured cells mutation level (%) |
|---------|-------------------|---------------------------|----------------------------------|
| 1 H1047R | Fatty tissue—left toe | 13 | — |
| 2 H1047R | Bone—left second toe | — | 28 |
| 3 H1047R | Skin—right third finger | 50 | — |
| 4 H1047L | Neural—right third finger | 5 | — |
| 5 H1047L | Skin—left 2nd toe | 5 | — |
| 6 [N68] H1047R | Skin—left second finger ulnar surface | — | <1–5 |
| 7 H1047R | Subcutis—left second toe | — | 17 |
| 8 [N110] H1047R | Skin—medial tip of second left toe | 7 | 31 |
| 9 ES42K | Skin piece 1—left foot | — | 29 |
| 10 [N104] H1047R | Skin—left hand | — | 2 |
| 11 H1047R | Skin—right great toe | 3 | 6 |
| 12 H1047L | Skin—left foot | — | 24 |
| 13 H1047L | Skin—left toe | — | 25 |
| 14 [N109] H1047R | Skin—dorsal left foot | 12–26 | 24–37 |
| 15 ES45K | Skin—right foot | 9 | — |
| 16 H1047R | Fibrofatty tissue—right side of abdomen | 1 | — |
| 17 [N99] H1047R | Growth plate—left second toe proximal phalanx | 31 | — |
|  | Growth plate—left second toe middle phalanx | 22 | — |
|  | Growth plate—left second toe distal phalanx | 25 | — |
|  | Growth plate—left third toe proximal phalanx | 27 | — |
|  | Growth plate—left third toe middle phalanx | 9 | — |
|  | Growth plate—left third toe distal phalanx | 14 | — |
|  | Skin—webbing between second and third toes | — | 33 |
|  | Bone—left talus | 3–6 | — |
|  | Bone—phalanx, right second toe | — | 4–10 |
|  | Nerve—left foot | — | 9 |
|  | Vessel 1—left foot | — | 25 |
|  | Vessel 2—left foot | — | 14 |
| Patient | Anatomical source | Tissue mutation level (%) | Cultured cells mutation level (%) |
|---------|------------------|---------------------------|----------------------------------|
| 18 H1047L | Blood | 0 | — |
| | Fibrofatty tissue—right foot | 39 | — |
| | Saliva | 0 | — |
| 19 H1047R | Nerve—left anterior tibial nerve | 7 | — |
| | Nerve—left posterior tibial nerve | 7 | 26 |
| | Adipose tissue piece 1—posterior left leg | 9 | 29–33 |
| | Adipose tissue piece 2—posterior left leg | 10 | 27–33 |
| | Connective tissue—posterior left leg | — | 4 |
| | Muscle—posterior left leg | 3 | 0–1 |
| | Tendon—posterior left leg | 0 | 0 |
| | Skin—posterior left leg | 4 | 8–22 |
| 20 H1047R | Skin—left forearm | — | 2 |
| | Skin—right forearm | — | 0 |
| | Lipoma—left forearm | 25 | — |
| | Blood | 0 | — |
| 21 [M001] H1047L | Adipose tissue—left leg | 39 | — |
| | Muscle—left leg | 33 | — |
| | Fibrous tissue—left leg | 32 | — |
| | Skin—left leg | 24 | 50 |
| | Bone—left leg | 8 | — |
| | Skin—left arm | — | 0 |
| | Blood | 0 | — |
| | Saliva | 0 | — |
| 22 [N7] H1047R | Articular cartilage—left foot | — | 33 |
| | Adipose tissue—left foot | — | 33 |
| | Bone—left foot | — | 33 |
| | Skin—left foot | — | 33 |
| | Muscle—left foot | — | 16 |
| | Deep tissue—left foot | — | 7 |
| | Blood | 0 | — |
| 23 [N108] H1047L | Skin—left leg | 3 | — |
| | Adipose tissue—left leg | 4 | — |
| | Blood | 0 | — |
| 24 ES45K | Lymphatic malformation—back | <1—1 | — |
| | Skin over lymphatic malformation—back | 0 | — |
| | Blood | 0 | — |
| 25 ES45K | Skin—right foot, second metatarsal | 7 | 12–15 |
| | Growth plate—right foot, second metatarsal | 11 | 15–17 |
| 26 H1047R | Skin, left buttock | — | 4 |
| | Skin, right upper inner arm | — | 0 |
| 27 [N45] H1047R | Skin—ankle | — | <1–2 |
| | Blood | 0 | — |
| 28 ES45K | Fat and fascia—left proximal tibia | 0 | 0 |
| | Fat and fascia—left distal femur | 1 | 3 |
| | Soft tissue—foot | 6 | — |
| | Growth plate—toe | — | 11 |
| | Normal skin—left tibia | 0 | 0 |
| 29 ES42K | Lymphatic malformation—left trunk | — | 5 |
| | Skin—left leg | — | <1 |
| 30 H1047R | Skin—abdomen | 7 | 0 |
| | Tonsil—left | 0 | 0 |
| | Tonsil—right | 0 | 0 |
| | Blood | 0 | — |
unilateral involvement of the orbit and cheek, and one had asymmetric overgrowth of the chest and torso. Two patients had involvement of the chest and torso, but not the limbs. The distal portion of the limbs was often the first to show observable overgrowth and subsequent progression more proximally. For example, multiple patients who initially presented with only macrodactyly of the toes had progression to the foot followed by involvement of the leg. The proximal portions of the limbs were almost never affected alone without involvement of distal structures. There was only one patient identified with only an affected leg and none with only an affected arm.

Twenty-one of 35 (60%) had unilateral overgrowth, with 15/21 (71%) being affected on the left side (binomial \( P = 0.078 \)). Twelve of 35 had bilateral involvement in which seven of the 12 had the left side more affected than the right (binomial \( P = 0.774 \)). Overall, the left was affected more than the right in 22/33 patients \( (P = 0.081) \). The tissues involved in the overgrowth were fibrous, lipomatous, vascular, and skeletal. Information regarding infiltration of lipomatous tissue into the muscle was available on 21 patients and occurred in 12/21 (57%) of these patients. Adipocellular investment into internal structures was identified in nine patients and involved the viscera (liver, spleen, pancreas), intestines, mediastinum, and spine (Fig. 3A–C).

Regional reduction of adipose tissue occurred in of 10 of 33 patients (30%) and involved the upper extremities and/or torso (chest/upper abdomen) in all these individuals, one of whom also had reduced adipose tissue of the leg unaffected by overgrowth (Fig. 3A1, A2 and B2, B4). Patient 22 had increase in subcutaneous fat in the areas where he previously had reduced adipose tissue after resection of areas of adipose overgrowth; he also maintained normal weight to height ratio despite having the multiple large masses.

None of the patients we report met the diagnostic criteria for Proteus syndrome [Biesecker, 2006], although all the patients met the general criteria, and some exhibited components of the three specific diagnostic criteria categories. Specifically, none had cerebriform connective tissue nevi (CCTN). A linear epidermal nevus was present in 4/35 patients (Fig. 4A and B), while an ovarian cyst was documented in two patients, and one individual had an unilateral ovarian cystadenoma. Dysregulated adipose tissue (either lipomatous lesions or regional lipohypoplasia) was seen in all patients, and there were 15/35 patients (43%) with vascular malformations, including capillary venous or lymphatic malformations. Some of these 15 individuals had combined venous/lymphatic malformations. However, there were no individuals with lung bullae, nor the Proteus syndrome facial phenotype.

Other limb findings included postaxial or preaxial polydactyly and cutaneous syndactyly, which involved only the toes. In particular, four had polydactyly: two with postaxial polydactyly (one unilateral left foot and one bilateral feet), one central and one with preaxial polydactyly (of the hallux). There were seven with cutaneous syndactyly: two with unilateral 2–3 toes, two with unilateral 2–4 toes, one bilateral with 2–5 toes (Right) and 2–4 toes (Left), and two unspecified (Fig. 5A–C).

Kidney abnormalities were reported in 11/26 (42%) of patients evaluated. These abnormalities included nephrogenic rests, pelvicystasis, dilated ureters, hydronephrosis, duplicated renal arteries, renal cysts, and enlarged kidney(s). One patient thought to have Wilms tumor by imaging instead had benign renal lesions (nephrogenic rests on pathological examination).
| Patient designation | 1 (M013) | 2 (N136) | 3 (M023) | 4 (M016) | 5 (M026) | 6 (N68) | 7 (N143) | 8 (N110) | 9 (N144) | 10 (N104) | 11 (N124) | 12 (N128) |
|---------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|----------|
| Mutation            | p.H1047R | p.H1047R | p.H1047R | p.H1047L | p.H1047R | p.H1047R | p.H1047R | p.H1047R | p.H1047R | p.H1047R | p.H1047R | p.H1047L |
| Original summary    | Macrodactyly | Macrodactyly | Macrodactyly | Macrodactyly | Macrodactyly | FAO | FAO | HHML | FAO | HHML | FAO | HHML |
| Age at time of      | 8 y      | 13 d     | 2 y      | 4.5 y    | 5 y      | 49 y     | 18 m     | 3 y      | 11 m     | 12 y 6 m | 32 y      | 7 y      |
| evaluation          |          |          |          |          |          |          |          |          |          |           |           |          |
| Age at onset of     | Birth    | Birth    | Birth    | Birth    | Birth    | Birth    | Prenatal | 2–3 m   | Birth    | Birth    | 1.5 y    | Prenatal |
| symptoms            |          |          |          |          |          |          |          |          |          |           |           |          |
| Sex                 | F        | F        | F        | M        | F        | M        | F        | M        | F        | F         | F         | F        |
| Sporadic            | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y         | Y         | Y        |
| Mosaic              | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y         | Y         | Y        |
| Epidermal nevus     | N        | N        | N        | N        | N        | N        | N        | N        | N        | N         | N         | N        |
| Ovarian            | N        | N        | N        | N        | N        | N        | N        | N        | N        | N         | N         | N        |
| cystadenoma(s)      |          |          |          |          |          |          |          |          |          |           |           |          |
| Testicular/         | N        | N        | N        | N        | N        | N        | N        | N        | N        | N         | N         | N        |
| epididymal          |          |          |          |          |          |          |          |          |          |           |           |          |
| abnormalities       |          |          |          |          |          |          |          |          |          |           |           |          |
| Asymmetric,         | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y         | Y         | Y        |
| disproportionate     |          |          |          |          |          |          |          |          |          |           |           |          |
| overgrowth          |          |          |          |          |          |          |          |          |          |           |           |          |
| Fibroadipose        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y        | Y         | Y         | Y        |
| overgrowth          |          |          |          |          |          |          |          |          |          |           |           |          |
| Affected areas of   | Lt great | Lt 2 toe | Lt 3, 4  | Lt 3, 4  | Lt 1, 2 toes | Lt 2 toe | Lt 2 toe | Lt 1, 2 toes | Lt 1–3 toes, foot | Lt 1 toe, foot | Lt 1, 2 toes |
| overgrowth [initial]| toe      | toe      | fingers  | fingers  | toes      | toe      | toe      | toes, foot   | toes      | toe, foot  | toes      |
| Lipomatous          | N        | N        | N        | NA       | N        | N        | N        | N        | NA       | NA        | NA        | NA       |
| infiltration of     |          |          |          |          |          |          |          |          |          |           |           |          |
| muscles             |          |          |          |          |          |          |          |          |          |           |           |          |
| Lipomatous          | N        | N        | N        | NA       | N        | N        | N        | N        | NA       | NA        | NA        | NA       |
| infiltration of     |          |          |          |          |          |          |          |          |          |           |           |          |
| internal structures |          |          |          |          |          |          |          |          |          |           |           |          |

[Continued]
| Patient designation | 1 (M013) | 2 (N136) | 3 (M023) | 4 (M016) | 5 (M026) | 6 (N68) | 7 (N143) | 8 (N110) | 9 (N144) | 10 (N104) | 11 (N124) | 12 (N120) |
|---------------------|----------|----------|----------|----------|----------|---------|----------|----------|----------|-----------|-----------|-----------|
| **Regional lipohypoplasia** (affected areas) | N | N | N | N | N | N | N | N | N | N | N | N |
| **Vascular malformations** (one or more) | N | N | N | N | N | N | Y | N | N | N | N | N |
| **Polydactyly** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Syndactyly** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Kidney abnormalities** | N | N | N | NA | N | NA | N | N | N | N | Y | ![Lt kidney enlg’d] |
| **Other malformations** | Rt ear microtia | N | N | Y [ganglion cyst] | N | N | Y [Rt preaur pt] | N | N | N | N | Y |
| **Other skin abnormalities** | N | N | N | Y [CA] | N | N | N | N | Y | Y | Y | Y-
| **Natural history features** | | | | | | | | | | | | |
| **General growth** | NA | 44/80 | 259–25 | >95/50 | 9–25/2–9 | 95/25 | 97/90 | 25/25 | 65–70/50 | 90/90 | NA | 25 birth |
| **OFC (centile)** | 50 | 29 | 25 | >95 | 4 | >97 | 75 | 90 | 25 | 60 | NA | NA |
| **Development** | NA | nl | nl | NA | NA | NA | nl | NA | nl | nl | NA | NA |
| **Progression of fibroadipose dysregulation** | Y: prog Lt 1 toe | Y: incr 1 toe | Y: prog Lt 3, 5 fingers | Y: prog incr 3, 4 fingers | Y: prog Lt 1, 2 toes | Y: enlg’d fingers to Lt palm | Y: to Lt 1 toe & foot | Y: to Lt 1 toe | Y: to Rt pen-umbilical & Lt foot | Y: to Lt thenar emin & 2 fingers | Y: to Lt 3 toe, foot & leg |
| **Surgeries** [remove overgrown tissue/amputations] | Y [1/0] | Y [1/0] | Y [0/2] | Y [2/0] | N | N | Y [2/1] | N | N | Y [3/0] | Yes, multiple | N |
| **Scoliosis** | N | N | N | N | N | N | N | N | N | N | N | N |
| Patient designation | 13 (N145) | 14 (N109) | 15 (M027) | 16 (M017) | 17 (N095) | 18 (M011) | 19 (N116) | 20 (N119) | 21 (M001) | 22 (N107) | 23 (N108) | 24 (N167) |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Mutation            | p.H1047L  | p.H1047R  | p.E545K   | p.H1047R  | p.H1047R  | p.H1047L  | p.H1047R  | p.H1047R  | p.H1047L  | p.H1047R  | p.H1047L  | p.E545K   |
| Original summary    | HHML      | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       |
| phenotype           | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       | FAO       |
| Age at time of evaluation | 4.5 y   | 5 y       | 2 y       | 32 y      | 9 y       | 5 y       | 49 y      | 27 y      | 7 y       | 7 y       | NB        |
| Age at onset of symptoms | Birth    | Birth     | Birth     | Birth     | Prenatal  | Birth     | Birth     | Birth     | Birth     | Birth     | Birth     |
| Sex                 | F         | F         | F         | M         | F         | M         | F         | M         | F         | M         | M         |
| Sporadic            | Y         | Y         | Y         | F         | M         | M         | F         | M         | F         | M         | M         |
| Mosaic              | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         |
| Epidermal nevus     | N         | N         | N         | N         | N         | N         | N         | N         | N         | N         | N         |
| Ovarian Cystadenoma[s] | N         | N         | N         | N         | N         | N         | N         | N         | N         | Ovarian cyst | N         | N         |
| Testicular/epididymal abscess | N         | N         | N         | N         | N         | N         | N         | N         | N         | Y [hydrocele] | N         | NA        |
| Asymmetric, disproportional overgrowth | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         |
| Fibroadipose overgrowth | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         | Y         |
| Affected areas of overgrowth [initial] | Lt foot, 2–4 toes | Lt 1 & foot | Bilat feet | Rt leg, abd, Lt foot | Bilat Lt 1–3, Rt 1, 2 toes, Lt > Rt leg | Rt leg | Lt 2–5 toes, foot, leg | Lt hand, 1, 2 fingers | Bilat feet & legs | Lt leg, buttack, foot | Rt foot, leg | Rt arm, hand |
| Lipomatous infiltration of muscles | Y         | NA        | NA        | NA        | NA        | N         | Y         | N         | Y [biopsy, nerves] | Y [biopsy, paraspinal] | Y [intrafascial RLD, Lt thigh] | NA |
| Lipomatous infiltration of internal structures | N         | N         | NA        | NA        | NA        | Y         | N         | N         | N         | NA        | T11-L4, pancreas | Lt intra-abdominal | Y |
| Patient designation | 13 (N145) | 14 (N109) | 15 (M027) | 16 (M017) | 17 (M99) | 18 (M011) | 19 (N116) | 20 (N119) | 21 (M001) | 22 (N7) | 23 (N108) | 24 (N167) |
|---------------------|-----------|-----------|-----------|-----------|---------|-----------|-----------|-----------|-----------|--------|-----------|-----------|
| Regional lipohypoplasia [affected areas] | N | N | N | N | N | Y | N | N | Y [upper body] | Y [upper body] | Y [upper torso, arms, face] | N |
| Vascular malformations | N | N | N | N | N | N | Y [prom. vasc. Lt calf] | Y [legs, feet] | N | N | Y [prom. vasc.] | Y [Lt thorax, neck, axilla] |
| Polydactyly | N | N | N | N | N | N | Y [Lt foot PA] | N | N | Y [Lt foot C] | N | N |
| Syndactyly | Y [2–4 toes] | Y [2–3 toes] | N | Y | Y [Lt pelvec] | N | N | Y [2–4 toes] | N | N | N |
| Kidney abnormalities | N | N | N | N | N | N | Y [enlg'd Rt, cysts] | N | N | Y [Lt pelvec] | NA | NA |
| Other malformations | N | N | Y | N | N | N | N | N | N | Y [DM 1] | N | CHOs |
| Other skin abnormalities | NA | N | Y [hypopig] | N | N | N | N | Y [PN, CM, hypopig] | N | N | N | Y [PN] | N |
| Natural history features | General growth [Wt/Ht CENTILE] | NA | 25/50 | 50/75 | >95/50 | NA | 60/50 | 80/75–90 | 90–95/50 | >95/50 | 50/50–75 | 70/37 | 90/95 |
| OFC [centile] | NA | 10 | ~25 | NA | NA | 50 | 75% | 30–40 | NA | NA | 91 | >95 |
| Development | ni | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Progression of fibroadipose dysregulation | Y: to Lt leg [prom. glut region], recurrence of Lt foot | Y: to Lt foot, Lt leg [prom. involvement buttock, lab maj] | Y: prog Bknt feet | Y: prog Bknt feet | Y: prog Bknt feet | Y: prog Bknt feet | Y: prog Lt leg, shoulder | Y: prog of legs, Rt abd | Y: prog of legs, gkt, vertebrae | Y: prog Lt leg, shoulder, abd | Y: prog of legs, Rt leg, foot | Y: prog Lt leg, buttock |
| Surgeries (remove overgrown tissue/amputations) | Y [1/1] | Y [2/1] | N | Y [1/0] | Y [2/0] | Y [6/2] | Y [4/2] | Y [6/3] | Y [3/1] | Y [~9/1] | Y [>3/0] | Y [1/0] |
| Scoliosis | N | N | N | N | Y | N | N | N | Y | N | N | N |
| Patient designation | 25 [N147] | 26 [N138] | 27 [N45] | 28 [N22] | 29 [N164] | 30 [N123] | 31 [N113] | 32 [N146] | 33 [M028] | 34 [N170] | 35 [M021] |
|---------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Mutation            | p.E545K  | p.H1047R | p.E545K  | p.E542K  | p.H1047R | p.E542K  | p.H1047R | p.C420R  | p.H1047R | p.H1047R | p.H1047R |
| Original summary phenotype | CLOVES | FAO | CLOVES | CLOVES | CLOVES | CLOVES | CLOVES | CLOVES | CLOVES | CLOVES | ENS |
| Age at time of evaluation | 7 y | 14.5 y | 31 y | 16 y | 4 y | 43 y | 46 y | 2 y | 8 m | 21 y |
| Age at onset of symptoms | Birth | Birth | Birth | Birth | Prenatal | ~1 y | Birth | Birth | Birth | Birth | Puberty |
| Sex                 | F | F | M | M | M | F | F | F | M | M | M |
| Sporadic            | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Mosaic              | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Epidermal nevus     | N | N | N | N | N | N | N | Y | Y | Y | N | Y |
| Ovarian cystadenoma(s) | N | N | N | N | N | N | Ovarian cyst | Y [unilateral] | N | N | N |
| Testicular/epididymal abnormalities | N | N | Y [hydrocele] | Y [hydrocele] | Blat hydrocele | N | N | N | N | NA | NA |
| Asymmetric, disproportionate overgrowth | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Fibroadipose overgrowth | Y | Y | Y | N | Y | Y | Y | Y | Y | N |
| Affected areas of overgrowth [initial] | Blat toes [Lt 1–5 Lt 2–4], feet | Blat feet Lt 1–5 Lt leg | Blat legs Lt > Rt leg; toes, abd; toes, leg | Blat 1–3 toes, Lt leg; toes, leg | Blat axilla, Lt scrotum, leg; Rt chest | Blat feet, legs Lt > Rt, enl’g’d fem, lipom spine, back, abd, chest | Rt foot, thigh, hand, tongue masses | Lt HH, Lt 4, 5 toes | Lt HH, Lt HM, Lt hemifacial | Back, abd | Multiple spinal tumors |
| Lipomatous infiltration of muscles | N | Y [muscles of legs, feet] | Y [blat legs, Rt buttock] | Y | Y | Y | Y | Y [Lt foot, T1-4 paraspinal] | Y [RLQ, Rt chest, Lt shoulder] | NA | N | N |
| Lipomatous infiltration of internal structures | Y | Y | Y [enl’g’d visc fat, bowel wall] | NA | Y | Y [interstital and spinal] | Y [liver, spleen, mediastinal] | Y | Y | N | N |
| Regional lipohypoplasia [affected areas] | N | Y [rest of body, Rt leg] | Y [UEs, torso] | N | NA | Y [chest and arms] | Y [Chest] | N | Y | Y [buttocks] | N |
| Vascular malformations [one or more] | Y | Y [prom veins of legs, abd, upper back] | Y [bowel] | Y [testes, cutan Lt chest] | Y | Y [blat prom leg veins] | Y [Lt abd, back, groin, mult hemang] | Y [Lt chest, abd, pelvis, mult LA of spinal cord, muscles, liver, spleen] | N | Y | N [large central artery in lipoma in post thoracic region] | (Continued)
| Patient designation | 25 (N147) | 26 (N138) | 27 (N45) | 28 (N22) | 29 (N164) | 30 (N123) | 31 (N113) | 32 (N146) | 33 (M028) | 34 (N170) | 35 (M021) |
|---------------------|-----------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Polydactyly         | N         | Y (Bilat PA feet) | N         | N         | N         | N         | N         | N         | N         | N         | Y         |
| Syndactyly          | N         | N         | Y (2–5 toes Rt; 2–4 toes Lt) | N         | N         | N         | N         | N         | N         | N         | N         |
| Kidney abnormalities| N         | Y [VUR, HN, enlg'd (Bil)] | Y [dilated coll system] | Y [Rt > Lt enlg'd] | Y [HN] | N         | Y [cysts] | N         | N         | Y [Bilat pelvec] | Y [dupic renal arts] |
| Other malformations | N         | Y [fused metatarsals, Rh hip sublux, elong vert] | N         | N         | Y [cerebral infants; vert abnorms] | Y         | Y [spina bifida occulta, bowel malrot, vertebral frad] | N         | N         | Y [tethered cord] | Y [Ectasia of aorta] |
| Other skin abnormalities | Y         | Y [hyper-pig with hair decr'd hair Lt leg] | Y. hypopig | NA        | N         | Y [hyperpig scalp, CM legs, Lt foot] | Y [CAL cutaneous blebs] | Y         | N         | N         | Y [mult EN; large lipoma on back] |
| Natural history features | 50/25 | 50/≤95 | 10–25/25–50 | 50/75 | 90/30 | 50/≤3 | >97/≤3 | NA | NA | >95/≤95 | NA |
| OFC                 | SO        | <3       | ~75      | 97       | 15      | <3       | 75–95/97 | NA | NA | 55       | >95     |
| Development         | nl        | NA       | NA       | Y/ADHD  | Gross motor delay | nl        | NA       | nl       | NA       | Delayed | NA |
| Progression of fibroadipose dysregulation | Y: prog macrodactyly and enlarged feet | Y: prog Lt leg foot, inc’d fatty infiltration in abd | Y: prog legs, feet, subcutan, intra-abd wall, viscera, bowel, irregular fem | Y: to Rt 3, 4 fingers, chest/back, Blat legs, feet [Lt > Rt] | Y: prog legs, feet, masses in chest, back, ab, spine | Y: prog legs, feet, masses in chest, back, ab, spinal r | Y: prog buttocks, feet, toes, leg [Lt > Rt] | Y: prog Lt leg, buttock, face, ear, tongue, gingiva | Y | N |
| Surgeries [remove overgrown tissue/ amputations] | Y [3/2] | N | Y [18/1] | Y [3–5/2] | Y [1/0] | Y [3/1] | Y [8–9/0] | Y (> 100) | Y [2/0] | Y [1/0] | Y [4/0] |
| Scoliosis           | Y         | Y         | N         | N         | N         | Y         | Y         | Y         | N         | N         | Y         |

<, less than; >, greater than; ab norm, abnormalities; ADHD, attention deficit hyperactivity disorder; aff, affected; arts, arteries; Bilat, Bilateral; CAL, café-au-lait macule; CLOVES, congenital lipomatosis, overgrowth, vascular malformations, and epidermal nevi, scoliosis/skeletal/spinal; coll, collecting; cong, congenital; CHDs, congenital heart defects; cutan, cutaneous; CM, cutis marmorata; de’d, deceased; DM 1, type 1 diabetes mellitus; dupic, duplicated; elong, elongated; emin, eminence; enlg’d, enlarged; ENS, epidermal nevus syndrome; FAO, fibroadipose overgrowth; fem, femoral head; F, female; glt, gluteus/gluta hemang, hemangiom; HN, hemihyperplasia; HM, hemimegalencephaly; HRML, hemihyperplasia multiple lipomatosis; HN, hydronephrosis; Ht, Height; hyperpig, hyperpigmented macule; hypopig, hypopigmented macules; inc, increase; lab maj, labia majora; Lt, Left; lipom, lipomatosis; LA, lymphangiomatosus; M, male; malrot, malrotation; mut, multiple; m, months; NA, not available; NB, newborn; NR, nephrogenic rests; nl, normal; N, no; OFC, occipito-frontal circumference; pelvec, pelvic axis; PN, pigmented nevus; PVNS, pigmented villonodular synovitis of right knee; PA, postaxial; prom, prominent; prog, progressive; Rt, right; RLQ, right lower quadrant of abdomen; subcutan, subcutaneous; sublux, subluxation; vasc, vasculature; vert, vertebral; VUR, vesicoureteral reflex; Wt, weight; y, year; Y, yes. Note that the centiles are approximate in many cases. Original Summary Phenotype is our diagnosis, prior to the recognition, from analyzing these data that all seem to be a single continuous phenotypic spectrum.

Key: Shading: original 8 cases Lindhurst et al. [2012].
Other skin abnormalities included a dermal melanocytic nevus, café-au-lait macules, hypopigmented macules, cutis marmorata, pigmented nevi, and patchy hyperpigmentation in 12/33 (36%) patients. Other malformations (minor and major) found in these patients (13/34 or 38%) included one patient with two cerebral infarcts (one was neonatal in onset), one with hemimegalencephaly, one with congenital heart defects (multiple VSDs, ASD), inguinal hernias, bowel malrotation, hip subluxation/dislocation, spina bifida occulta, tethered cord, extra segments in the vertebrae, uterine fibroids, and splenic cysts, and the remainder with minor anomalies including one with ganglion cyst and one with right preauricular pit. One patient had type 1 diabetes mellitus. Spinal and major nerve neurofibromas were also reported in two patients. Patient 35 had biopsy-proven spinal neurofibromas removed at several levels; \textit{NF1}, \textit{NF2}, and \textit{PTEN} genes were also tested and were normal. These patients did not have other manifestations of Neurofibromatosis, types 1 or 2. In Patient 21, her lumbosacral plexus had multiple nodular lesions on MRI that have been identified malignancies in these patients.

Fifteen had amputations of the affected leg or digits. In multiple patients, there was continued growth in the affected limb after surgical amputation.

**DISCUSSION**

The recent finding of \textit{PIK3CA} mutations in a spectrum of overlapping forms of overgrowth affords the opportunity to gain insight into the pathophysiologic basis of these conditions, and suggests that a reappraisal of current clinical classification is timely. This study provides a clinical and molecular evaluation of 35 patients with \textit{PIK3CA} somatic mutations.

**Novel Findings**

Novel overgrowth findings in these patients included: adipose dysregulation present in all patients, unilateral overgrowth that was predominantly left-sided, overgrowth that affected the lower extremities more than the upper extremities and progressed in a distal to proximal pattern, and in the most severely affected patients was associated with marked paucity of adipose tissue in unaffected areas. While not statistically significant, when overgrowth was asymmetric, it was often left-sided. Larger patient numbers are needed to assess whether this is significant. The underlying mechanism for the observed distal to proximal pattern of progression of overgrowth with only one patient showing earlier proximal involvement is unknown at present. There also was statistically significant association of genotypes with phenotypic groupings within the spectrum of \textit{PIK3CA} somatic mutations. All but two of the patients with the phenotype most consistent with either FAO, HHML, or macrodactyly designations had a mutation in the catalytic domain (codon 1047), while the majority of patients with the CLOVES syndrome designation had mutations in the coiled domain with a \(P\) value of 0.0014 (Fig. 1).

**Clinical Classification**

The previously reported phenotypic descriptors in patients with \textit{PIK3CA} somatic mutations included FAO [Lindhurst et al., 2012], HHML [Biesecker et al., 1998] and CLOVES syndrome [Sapp et al., 2007; Alomari, 2009; Kurek et al., 2012], isolated macrodactyly [Rios et al., 2013], and the megalencephaly syndrome, MCAP [Rivière et al., 2012]. The present study focused on patients with non-CNS phenotypes. Patients previously diagnosed with FAO, HHML, CLOVES syndrome, and isolated macrodactyly [Lindhurst et al., 2012; Kurek et al., 2012; Lee et al., 2012; Rios et al., 2013] had considerable overlap and we were unable to discern a rational boundary that would separate FAO, HHML, or macrodactyly. In all three, there was congenital, static, or mildly progressive asymmetric
FIG. 2. Spectrum of overgrowth in patients with somatic PIK3CA mutations (A) Patient 2 at 13 days of age with macrodactyly of the left second toe; (B) Patient 7 at 18 months of age (1) Dorsal view of the left foot with overgrowth following surgical resection of T2, (2) Ventral views of both feet show overgrowth of the middle to distal ventral region of the left foot; (C) Patient 9 at 11 months of age (1–3) Enlargement of the left T1–3 with ballooning appearance of the distal portion, and increased circumference and length of the entire left foot, (4) X-ray of the left foot shows enlarged phalanges of T1–3; (D) Patient 15 (1) At 1 day of age, an enlarged right foot, (2) At 18 months of age, medial deviation and progressive widening of both feet, (3) Areas of hypopigmentation on the legs and (4) back, (5) T1-weighted MRI scan shows a cystic lesion adjacent to the lumbar spine; (E) Patient 19 at 5 years 6 months of age (1) Frontal view shows overgrowth of the left leg following trans-tibial amputation at four years of age, (2) Posterior view of the legs, (3) X-ray of the legs shows an enlarged left femur, tibia and fibula; (F) Patient 20 at 49 years of age (1) Enlarged left shoulder, arm and hand: left F4, 5 appeared normal, left F2,3 are missing following surgical amputation, and there is a 2 cm lipoma between the PIP and DIP joints of F4, (2) Left F1 is enlarged and surgically repositioned, (3) X-rays show her hand following surgical resection of F2, 3 and an enlarged left humerus; (G) Patient 28 at 10 years of age (1 and 2) Enlargement of the feet and legs, more severe on the left, (3) Enlargement and angular deformity of the right F3, 4, (4) X-rays of the hands show bone and soft tissue overgrowth of the phalanges of the right F3, 4, (5) X-rays of the feet: left foot shows four toes following surgical resection of T2, 3 and overgrowth of the metatarsals, the right foot shows four toes with absent T2, overgrowth of metatarsals and phalanges of T1 and T3, bony fusion with an “H” configuration of metatarsals of T1 and T3, and small second cuneiform.
overgrowth associated with areas of increased subcutaneous adipose tissue. This study included four patients who had only macrodactyly, although this macrodactyly was also associated with overgrowth of adipose tissue.

The CLOVES syndrome manifests prenatal asymmetric overgrowth that is primarily proportionate in nature [Sapp et al., 2007; Alomari, 2009]. Affected persons commonly had splayed feet and toes. The vascular malformations were most commonly combined lymphatico-venous anomalies with cutaneous blebbing and weeping. The lipomatous nature of the overgrowth was characterized by overgrowth of fat within normal fatty fascial planes and linear verrucous epidermal nevi. CLOVES syndrome has also been associated with CNS abnormalities [Sapp et al., 2007; Alomari, 2009]; there was only one patient with hemimegalencephaly confirmed in this series. The patients described here were previously designated as having CLOVES syndrome if they had congenital lipomatous overgrowth, vascular malformations, and skeletal/spinal involvement with or without the presence of the epidermal nevi. Three of nine patients with the phenotype of CLOVES syndrome had epidermal nevi. One of the four patients in this study with clinical findings of Epidermal Nevus Syndrome had epidermal nevi. On

anterior tibia, and an enlargement and bowing of the left fibula, subluxation with shallow acetabulum of the right hip, and overgrown and elongated right fibula and greater trochanter, [8] X-rays of the spine show scoliosis of the mid-thoracic spine centered at T6, and elongated and overgrown lower thoracic and lumbar vertebral bodies; [B] Patient 30 at 4 years of age [1] Posterior view of her whole body shows multiple lipomatous lesions involving her abdomen, chest and back, and upper body lipohypoplasia, [2] Views of her legs and feet show asymmetry of the leg positioning and size, widened feet with splayed toes with the left side larger than the right, and the left leg with prominent superficial veins, [3] Lateral view shows her barrel-chest and prominent abdomen with multiple lipomatous masses involving her abdomen and back, [4] Frontal view shows her chest and arms with lipohypoplasia, and protuberant abdomen with multiple lipomatous masses, [5] MRI scan of the lateral lower thoracic, lumbar and sacral vertebrae shows multi-level foraminal soft tissue masses, some of which are associated with dural ectatic changes, and subcutaneous soft tissue masses most compatible with the lipomas involving the back, and the extra segmentation in coccyx, [6] X-ray of the pelvis and upper legs shows right hip dislocation, abnormal acetabulum and femoral head; [C] Patient 31 at 43 years of age [1] Posterior view of her head, back and abdomen shows multiple masses and scars on her back from surgical excision of the venolymphatic malformations, and an epidermal nevus on her right cheek and pinna, [2] X-ray of the spine shows thoraco-lumbar scoliosis following surgical rod placement, [3–5] MRI scans of the chest, abdomen, and pelvis show scoliosis, marked fatty intermixure of the paraspinal muscles, splenomegaly with multiple cysts, irregular enhancement of the muscles of the posterior chest wall on the left, obesity with marked intra-abdominal fat, periaortic and paracaval foci compatible with additional collateral vessels or small vascular masses, and grossly normal caliber of the superior and inferior vena cava, the thoracic and abdominal aorta, and the common iliac vessels.

Fig. 3. Spectrum of overgrowth in patients with somatic PIK3CA mutations. A: Patient 26 at 14 years 6 months of age [1] Frontal view shows the upper body lipohypoplasia and bilateral leg overgrowth, more severe on the left, [2] Dorsal view of the feet show bilateral postaxial polydactyly with more severe overgrowth of the left foot, [3] X-rays of the feet: the right foot shows marked overgrowth of the metatarsals of T1—4, unusual epiphyses of all rays, and a supernumerary metatarsal, the left foot shows six toes with an enlarged metatarsal of T1 and proximal fusion of the metatarsal bones, [4] MRI of the lateral spine shows a large mass over her upper thoracic region; multiple neural foraminal masses at several levels in the thoracic spine, some of these are associated with dural ectatic changes and prominent CSF in the nerve root sheath, [5] MRI scan of the legs shows extensive soft tissue overgrowth, primarily in the fatty tissues with fat tissue intermixed with muscle in the left leg, [6 and 7] X-rays of the pelvis and legs show marked asymmetry and enlargement of the left hemipelvis and leg, completely dislocated left hip with unusual overgrowth pattern of the lesser trochanter and proximal femur, an enlarged left distal femur, particularly the medial femoral condyle, a displaced left patella, an abnormal configuration of the left
reviewing all of the patients here, we conclude that it may be difficult to distinguish those patients described as having CLOVES syndrome from more severe FAO/HHML if they have an epidermal nevus or vascular malformations.

We conclude that the phenotypic descriptors of FAO, HHML, and macrodactyly associated with PIK3CA mutations are not sufficiently distinct to warrant separate clinical descriptors. Further, the descriptor of CLOVES syndrome may reside at an extreme of the spectrum formerly including FAO, HHML, and macrodactyly.

Many of these patients had previous diagnoses including Klippel–Trenaunay syndrome (KTS) and Proteus syndrome, however, they did not meet published diagnostic criteria for either condition. Once again, however, there was overlap of findings in these patients with those disorders. Klippel–Trenaunay syndrome manifests both overgrowth and vascular malformations. However, in KTS the
overgrowth is generally ipsilateral and overlapping with the vascular malformations. The typical vascular malformation is the lateral venous anomaly, and the skeletal overgrowth lacks the distortion and progressivity seen in persons with Proteus syndrome [Biesecker et al., 1998; Cohen, 2000], and the patients reported here. Moreover, the patients currently described lack the hallmark skin finding of Proteus syndrome (CCTN), as noted in Table II.

Genotype–Phenotype Correlation

Our data suggest that some genotype–phenotype correlation may exist, that is, there are recognizable patterns of overgrowth associated with the five different mutations identified in these 35 patients. The most frequently identified PIK3CA mutation was in codon 1047 (27 patients), and the predominant feature in patients with that mutation was a progressive, mosaic phenotype of FAO with other areas of deficient adiposity in those with severe overgrowth, but less frequently associated with vascular malformations. Of the 27 patients with codon 1047 mutations, 19 had p.His1047Arg substitution and eight had p.His1047Leu substitution. Of these 27 patients, 14 were previously diagnosed by us as having FAO, five were diagnosed with HHML, four were diagnosed with macrodactyly, three were diagnosed with CLOVES syndrome, and one with possible Epidermal Nevus Syndrome.

Those having a phenotype more compatible with CLOVES syndrome had a mix of less frequently observed mutations, including p.Glu542Lys, p.Glu545Lys, and p.Cys420Arg, as well as p.His1047Arg, similar to the six patients reported by Kurek et al. [2012]. Genetic studies of further patients with CLOVES syndrome may provide a better understanding of the distribution of causative somatic mutations within PIK3CA.

The mutations within PIK3CA were detected in affected tissues or cultured cells at varying levels, but not detected in the blood (in 21 patients) or saliva (in three patients). There was not a clear correlation of mutation level in either tissues or cultured cells to either the quality (nature) of the manifestations or the overall severity of the manifestations. Patient 1, who was considered to be mildly affected, had a mutation burden of 31% in the sampled affected tissue, whereas in Patient 21 the mutation burden was only 7% in the sampled tissue. We hypothesize that the overall lack of correlation of severity to mutation burden emanates from the severe sampling limitations. Our ability to sample tissues is limited both by human subjects considerations and practicality. Indeed, we predict that in the more mildly affected patients, the many unaffected areas of their bodies would show a low or zero level of the mutation, which would contrast with patients who had extensive areas of overgrowth. In contrast to our results showing an association of keratinocyte versus fibroblast mutation level with the nature of the cutaneous manifestations of Proteus syndrome [Lindhurst et al., 2014], the present study only assayed fibroblasts from biopsies.

Increased and Decreased Adipose Tissue

Some patients had striking lipoatrophy in areas not affected by overgrowth, which occurred in those who had more severe overgrowth. Further, this finding was more common in patients with CLOVES syndrome (4/9, 44%) or more severe manifestations of FAO/HHML (6/21, 28%). Interestingly, in one patient (Patient 22), when the overgrown adipose tissue was resected, there was increased deposition of fat in the areas with previously decreased adipose tissue. These observations raise questions about the role of PI3K signaling in regulation of body fat deposition. Lindhurst et al. [2012] suggested that the adipose tissue paucity in the non-overgrown areas of the patients is caused by chronic negative energy balance of adipose depots consequent to the demands of the pathologically growing and energy-sequestering adipose tissue in affected regions.

PI3K signaling activates the serine/threonine kinases AKT1, AKT2, and AKT3. AKT1 is most widely expressed, and is associated with growth [Chen et al., 2001], consistent with the Proteus phenotype, while AKT2 is highly expressed in insulin-responsive tissues including skeletal muscle, liver, and fat, and is more closely implicated in the metabolic actions of insulin [Whiteman et al.,
muscle or visceral tissue. The nature of the overgrown tissue was fibroadipose tissue rather than from enlargement of the actual secondary enlargement; therefore, the overgrowth primarily was in fibroadipose tissue into muscle and visceral organs often causing documented prenatally in four. Often, there was infiltration of the Onset of overgrowth in the majority of patients was congenital and Natural History One hypothesis is that the patterning and involvement of the PI3K/AKT signaling pathway. Not known. However, this finding points to an early defect in limb development or agenesis has been reported in CLOVES syndrome, but not in those with FAO or macrodactyly [Alomari, 2009; Kurek et al., 2012; Lindhurst et al., 2012].

Other Characteristic Associated Findings Polydactyly and/or cutaneous syndactyly was seen in nine patients and exclusively involved the toes with variable pattern of involvement. The pattern of cutaneous syndactyly involved toes 2–3 and 2–4 most commonly. The frequency and pattern of polydactyly and cutaneous syndactyly in these patients suggests this is a manifestation of this spectrum of disorders. The mechanism for this finding is not known. However, this finding points to an early defect in limb patterning and involvement of the PI3K/AKT signaling pathway. One hypothesis is that the PI3K gene interacts with other genes involved in limb patterning, including GLI3. Interaction of PI3K and AKT1 with GLI3 has been demonstrated in a novel KRAS-initiated pathway leading to VMP1 in cancer cells [Lo Re et al., 2012]. Alternatively, we speculate that the overgrowth of the feet in these patients may interact with normal patterning signals and gradients, but produce polydactyly due to the increased size of the limb. In support of this hypothesis, studies by Bouldin and Harfe [2009] using the Dorking chicken mutant found that over-proliferation due to FGF signaling caused polydactyly. In addition Lu et al. [2005] found that over-expression of Fgf4 resulted in poly-syndactyly in the mouse. As FGF signaling is not a primary determinant of anterior–posterior patterning and is instead a determinant of AER size and limb growth, we suggest that activation of the AKT/PIK3CA pathway analogously increases AER and/or limb bud size.

Macrocephaly (OFC ≥ 90th centile) was present in 30% of patients in this study. There was only one patient with a central nervous system abnormality, hemimegalencephaly, but this is likely attributable to our ascertainment bias.

Urinary and kidney abnormalities were found in approximately 40% of the patients; however renal function was normal. Renal underdevelopment or agenesis has been reported in CLOVES syndrome, but not in those with FAO or macrodactyly [Alomari, 2009; Kurek et al., 2012; Lindhurst et al., 2012].

Natural History Onset of overgrowth in the majority of patients was congenital and documented prenatally in four. Often, there was infiltration of the fibroadipose tissue into muscle and visceral organs often causing secondary enlargement; therefore, the overgrowth primarily was in fibroadipose tissue rather than from enlargement of the actual muscle or visceral tissue. The nature of the overgrown tissue was best exemplified by patient 19, where serial sections of an amputated leg show that this limb was almost entirely fibroadipose tissue, but also demonstrated radiographically in patients 17 and 26. Furthermore, the overgrowth was progressive in all patients, in size and sometimes also in location with spread involving adjacent areas. However, bilateral involvement did not correlate with the age of the patient at evaluation, suggesting that bilateral manifestations are not simply due to age.

Treatment of segmental overgrowth disorders has relied upon surgical debulking [Biesecker, 2006] and orthopedic procedures to limit growth [Tosi et al., 2011]. The majority (83%) of these 35 patients had surgical interventions for their overgrowth, many with multiple surgeries including 43% with amputations of affected limbs and or digits. These interventions occurred throughout the patients’ early childhood and into adulthood. It is clear from the patients presented herein that there is marked variability in rate of progression and number of complications. More longitudinal clinical data are needed regarding natural history on the effects of rate of overgrowth at different ages and after surgical debulking.

Potential Tumorigenesis and Cancer Risk and Surveillance Recommendations While there were no identified malignancies in these patients, two patients had tumors, one with potential premalignant findings of nephrogenic rests, and another with ovarian cystadenoma, which has not been reported previously. In addition, Kurek et al. [2012] reported one patient with p.His1047Arg mutation having Wilms tumor. The catalytic subunit of phosphatidylinositol-3-kinase (PI3K) is somatically mutated in many cancers including colorectal, ovarian, breast, and hepatocellular carcinomas, and in glioblastomas [Vivanco and Sawyers, 2002; Campbell et al., 2004; Lee et al., 2005; Levine et al., 2005; Li et al., 2005; Velho et al., 2005; Yuan and Cantley, 2008]. These PIK3CA mutations were located mostly at hotspots within the helical domain (encoded by exon 20), and they resulted in gain of function mutations that were implicated in oncogenicity [Samuels et al., 2004; Ikenoue et al., 2005; Kang et al., 2005]. Recently, Cizkova et al. [2013] found that patients with HER2-positive breast cancer, having PIK3CA mutation positive tumors, which were treated with trastuzumab, had a worse outcome than those with wild-type tumors. Given the prevalence of PIK3CA codon H1047 mutations in cancer, a critical consideration is whether patients with these mutations are at increased risk of malignancy. Transgenic expression of the Pik3ca p.His1047Arg mutation in lung [Engelman et al., 2008], or breast epithelium [Adams et al., 2011; Meyer et al., 2011] in mice has been shown to produce malignant tumors. However, in these studies mutant Pik3ca was overexpressed, potentially exaggerating its oncogenicity. Expression of Pik3ca p.His1047Arg at endogenous levels in mouse ovaries did not produce tumors after 1 year [Kinross et al., 2012]. It is possible that expression at endogenous levels in the cellular context of human mesodermal lineages has more benign consequences than implied by the mouse models overexpressing mutant Pik3ca. It is of note that codon 1047 oncogenic PIK3CA mutations are common in benign seborrheic keratoses and epidermal nevi in humans [Hafner et al., 2007], demonstrating that there is no obligate association of these mutations to malignancy. However, longitudinal studies are
needed to properly assess this potential risk and to formulate surveillance recommendations, should such a risk be identified.

Current recommendations for tumor surveillance are based upon a reported Wilms tumor in a patient with CLOVES syndrome [Kurek et al., 2012] and of nephrogenic rests (a premalignant tumor) in one of the patients reported here. Although the evidence is not sufficient to demonstrate high risk, it may be prudent to consider serial abdominal ultrasounds every 3–4 months until age 8 years in all patients with a somatic PIK3CA mutation similar to the recommendations in isolated hemihyperplasia and Beckwith–Wiedemann syndrome. In addition, because of the finding of spinal root and major nerve neurofibromas, as well as lipomatous lesions involving the spine, neurological monitoring, and spinal MRI scan should be considered in patients with truncal involvement. Finally, a reported risk of pulmonary embolism in patients with CLOVES syndrome having thoracic and central phlebectasia [Alomari et al., 2010] and as presented in this series, spinal thrombosis in patient 21 and neonatal cerebral infarcts in patient 30 suggest that it is important to be aware of the possible associated thrombosis risk in this group of patients. It is known that the related disorder, Proteus syndrome also has an increased risk of thrombosis, and consideration of anticoagulant prophylaxis is recommended in patients undergoing surgery or other procedures that may predispose to deep venous thrombosis or pulmonary embolism.

These patients should be monitored for other potential associated complications, including vascular malformations and skeletal and spinal abnormalities. More specific recommendations for surveillance will be forthcoming based upon analyses in a larger population of patients with PIK3CA somatic mutations.

Implications for Design of Future Therapeutic Trials

The results of this study highlight the need to collect specific clinical data prospectively to design future clinical trials. Clinical information regarding the assessment of cosmetic and functional parameters affected by the overgrowth, including but not limited to mobility, extent of vascular malformations and its associated risks, ventilation and metabolic status is essential in these patients to understand natural history fully, as well as to evaluate treatment effectiveness. Future targeted therapies may be possible with the identification of activated PI3K/AKT signaling, either through inhibition of PI3K, of AKT, or of downstream pathways such as mTORC1, using clinically available drugs. Rapamycin was reportedly beneficial in a child with type II segmental Cowden syndrome associated with PTEN deficiency [Marsh et al., 2008]. Patients with colorectal cancer and tumor positive for PIK3CA mutations, who are treated with aspirin may have prolonged survival [Ogina et al., 2013; Pintz, 2013; Sahin and Garrett, 2013; Viudez et al., 2013]. Intensive efforts are underway to develop novel inhibitors for use in cancer. The progressive nature of this disorder makes it a good target for pharmaceutical therapy because downregulation of the pathway may prevent the disease progression that is seen in many of the patients reported here.

In conclusion, based upon the results of this clinical and molecular analysis of 35 patients, we propose that the clinical entities formerly described as FAO, HHML, macrodactyly, and CLOVES syndrome caused by PIK3CA somatic mutations represent a single phenotypic spectrum. CLOVES syndrome represents a more severe subset of that spectrum. In addition, previous authors [Lee et al., 2012; Mirzaa et al., 2012; Rivière et al., 2012] have described the megalencephaly syndromes that have overlapping findings with CLOVES, FAO and HHML. Therefore, we propose the phenotypic designation of PIK3CA-Related Overgrowth Spectrum. While Mirzaa et al. [2013b] proposed a similar designation, “PIK3CA-related segmental overgrowth”, our designation is distinct for the following reasons: (1) the absence of the term “segmental” because there are patients having the PIK3CA somatic mutation who present with bilateral and systemic involvement, and (2) the inclusion of the term, “spectrum” to emphasize that there are different but related phenotypes rather than one specific phenotype. There is evidence of a correlation of genotype and phenotype, with CLOVES syndrome associated with coiled domain mutations and the FAO/HHML/macrodactyly phenotype associated with mutations in the catalytic domain. The overgrowth findings most commonly involve the lower extremities. Our data also suggest that the distal limb is affected more often than is the proximal segment, and with progression involves more proximal structures. Other characteristic associated findings include polydactyly (all types) and cutaneous syndactyly (together or separately), kidney and urinary tract abnormalities, and occasionally, abnormalities of the ovaries (cysts) and testes (hydroceles). Longitudinal studies of larger cohorts are needed to determine the rate and extent of bony and muscular involvement, as well as the pathogenetic mechanisms causing the distinct manifestations associated with somatic PIK3CA mutations. We recommend testing for the PIK3CA mutations on affected tissues in a patient presenting with any of the key features described herein.

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