**PIK3CA-Related Overgrowth Spectrum (PROS): Diagnostic and Testing Eligibility Criteria, Differential Diagnosis, and Evaluation**


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**Abstract**

Somatic activating mutations in the phosphatidylinositol-3-kinase/AKT/mTOR pathway underlie heterogeneous segmental overgrowth phenotypes. Because of the extreme differences among patients, we sought to characterize the phenotypic spectrum associated with different genotypes and mutation burdens, including a better understanding of associated complications and natural history. Historically, the clinical diagnoses in patients with PIK3CA activating mutations have included Fibroadipose hyperplasia or Overgrowth (FAO), Hemihyperplasia Multiple Lipomatosis (HHML), Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome, macrodactyly, Fibroadipose Infiltrating Lipomatosis, and the related megalencephaly syndromes, Megalencephaly-Capillary Malformation (MCAP or M-CM) and Dysplastic Megalencephaly (DMEG). A workshop was convened at the National Institutes of Health (NIH) to discuss and develop a consensus document regarding diagnosis and treatment of patients with PIK3CA-associated somatic overgrowth disorders. Participants in the workshop included a group of researchers from several institutions who have been studying these disorders and have published their findings, as well as representatives from patient-advocacy and support groups. The umbrella term of “PIK3CA-
Related Overgrowth Spectrum (PROS)” was agreed upon to encompass both the known and emerging clinical entities associated with somatic PIK3CA mutations including, macrodactyly, FAO, HHML, CLOVES, and related megalencephaly conditions. Key clinical diagnostic features and criteria for testing were proposed, and testing approaches summarized. Preliminary recommendations for a uniform approach to assessment of overgrowth and molecular diagnostic testing were determined. Future areas to address include the surgical management of overgrowth tissue and vascular anomalies, the optimal approach to thrombosis risk, and the testing of potential pharmacologic therapies.

Keywords
Somatic mosaicism; PIK3CA gene; fibroadipose overgrowth; segmental overgrowth; macrodactyly; CLOVES syndrome; PIK3CA-Related Overgrowth Spectrum (PROS)

INTRODUCTION
Over the past 15 years, substantial efforts have been invested in the clinical delineation of mosaic or segmental overgrowth disorders [Cohen, Neri, Weksberg, 2002]. The entity of macrodactyly and macrodystrophia lipomatosa has long been established in the clinical literature [Ho et al., 2002; Rios et al., 2012]. There has been confusion and controversy regarding the diagnosis of Proteus syndrome and PTEN-related overgrowth conditions [Eng, 2001, 2003; Biesecker et al., 1999]. Biesecker et al. [1998] described a segmental overgrowth condition termed hemihyperplasia with multiple lipomatosis (HHML). Sapp et al. [2007] delineated an apparently distinct recognizable entity called CLOVE syndrome (Congenital Lipomatous Overgrowth, Vascular Malformations, and Epidermal Nevi) as a distinct disorder from Proteus syndrome. Lindhurst et al. [2011] determined that the molecular defect in Proteus syndrome was a somatic mosaic gain-of-function mutation in AKT1, and Hussain et al. [2011] linked AKT2 activating mutations to overgrowth and hypoglycemia. In 2012, five papers were published linking the PIK3CA, PIKR2, AKT3, and other genes to somatic overgrowth and brain anomalies in humans [Lindhurst et al., 2012, Lee et al, 2012, Poduri et al., 2012; Rios et al., 2012; Riviere, et al, 2012; Kurek et al., 2012] (Figure 1).

The identification of PIK3CA somatic mutations was confirmed in patients with distinct, but partially overlapping clinical findings [Keppler-Noreuil et al., 2014], which have included Fibroadipose hyperplasia or Overgrowth (FAO) [Lindhurst et al., 2012], HHML [Biesecker et al., 1998], CLOVES syndrome [Sapp et al., 2007; Alomari, 2009; Kurek 2012], macrodactyly and muscle hemihypertrophy [Rios et al., 2013], the related megalencephaly syndromes, Megalencephaly-Capillary Malformation (MCAP) [Riviere et al., 2012], and hemimegalencephaly [Lee et al., 2012], skin disorders including benign lichenoid keratosis (BLK) [Groesser et al., 2012], and epidermal nevi (EN) and seborrheic keratosis (SK) [Hafner et al., 2007], and Fibroadipose Infiltrating Lipomatosis [Macelllan et al., 2014] (Figure 2). The distinct clinical focus of the investigators has led to ascertainment bias in the published literature, complicating a comprehensive assessment of genotype-phenotype correlation analysis. We set out to reconcile our current understanding of these phenotypes.
to provide a more clear understanding of the range in degree of severity of these mosaic disorders and their relationship to particular mutations within PIK3CA, and the level and anatomical distribution of mosaicism within this gene. It was our objective to resolve these apparently conflicting and overlapping designations to provide clarity in research endeavors and to give clinicians useful designations for management, prognostication, and the design of clinical trials of targeted therapies.

**DISCUSSION**

**GOALS & OBJECTIVES**

A two-day workshop was convened on the NIH campus in Bethesda, Maryland, on September 11 and 12, 2013 to discuss emerging clinical and molecular information on the phenotypes caused by somatic mutations in the PIK3CA gene. The initial goals of the meeting were to: (Text box 1).

The participants included several researchers who have been studying this group of disorders and three parent representatives of patient-family support and advocacy organizations for individuals with these conditions. A representative participant from each research group presented data to address the objectives of the meeting [Table I].

Following these presentations, the objectives for the remainder of the workshop were agreed upon and included delineation of key diagnostic features to guide molecular/ genetic analysis, eligibility criteria for molecular testing, and preliminary consideration of available technical approaches to testing. Preliminary recommendations for a uniform approach to a clinical evaluation of PROS were determined.

**CLINICAL DIAGNOSTIC CRITERIA**

Because of the phenotypic variability of these disorders, the group considered, and then designated a diagnostic umbrella or syndrome family [Brunner and van Driel, 2004] name for these disorders: PIK3CA-Related Overgrowth Spectrum (PROS). The proposed clinical diagnostic criteria for PROS are shown in Table II. While there is a spectrum of findings, we designated required characteristics that we felt were relevant across all of the entities. The first indications were based on the known natural history of PROS, which is that they are congenital or have early childhood onset. To date, all patients with PROS have been sporadic (no family history) and all have had mosaic distribution. While most patients have a progressive course, this is not a mandatory finding, and patients may manifest a spectrum or isolated features as outlined in Table II. The diagnostic criteria were subdivided into two major categories: category A with a diverse mosaic spectrum involving two or more of the listed features, and category B with one isolated and more tissue specific feature. Affected individuals may have one or more findings from Category A. Individuals may have only one of the listed findings in Category B, but for these manifestations to satisfy this criterion, they must be congenital or early childhood in onset. These criteria are intended to aid clinicians evaluating patients and to support research and clinical studies. It was the consensus of the group that the criteria should be simple, yet as comprehensive as possible given the current
state of knowledge. The group acknowledged that these criteria may require revision as the
gene mutations and clinical findings are further characterized.

SPECTRUM OF PIK3CA-RELATED OVERGROWTH

The PIK3CA-Related Overgrowth Spectrum (PROS) encompasses all the unique clinical
entities, but highlights the continuum and overlap between the diagnoses, as illustrated in the
Venn diagram in Figure 3. We anticipate that PROS will replace the previous component
clinical entities caused by somatic PIK3CA mutations, similar to, for example, the use of the
term “22q11.2 deletion syndrome”, which encompasses and has replaced the previous
clinical syndromes of DiGeorge syndrome, Velocardiofacial syndrome, Shprinzten
syndrome, Cayler cardiofacial syndrome, and Conotruncal anomaly face syndrome. In
PROS, there is varying severity in the clinical presentation of the patients, and some appear
to have tissue-specific distribution, while others are more pleiotropic. Macrodactyly (type I
macrodactyly or lipofibromatous hamartoma of nerve), also known as macrodystrophia
lipomatosus, was characterized by fibrofatty tissue enlargement and bony overgrowth,
typically within a ‘nerve territory’ with enlargement in circumference and length of the
peripheral nerve [Ho et al., 2002; Rios et al., 2012]. There is also a subset of patients with
macrodactyly who also have muscular hemihyperplasia. The original descriptions of
HHML, FAO, and macrodactyly have significant overlap and are difficult to discriminate
from one another. The HHML designation was originally described as moderate
abnormalities of asymmetry and overgrowth with multiple subcutaneous lipomata, and the
hemihyperplasia may be static or mildly progressive [Biesecker et al., 1998]. The major
manifestation of FAO was segmental and progressive overgrowth of subcutaneous and
visceral fibroadipose tissue, sometimes associated with skeletal and muscular overgrowth
[Lindhurst et al., 2012]. Recently, patients with facial infiltrating lipomatosis, characterized
by hemifacial soft-tissue and skeletal overgrowth, precocious dental development,
macrodontia, hemimacroglossia, and mucosal neuromas were diagnosed with PIK3CA
activating mutations [Macelllan et al., 2014]. There is also significant overlap of HHML and
FAO findings with some cases of CLOVES syndrome. As described above, CLOVES
syndrome was characterized by congenital lipomatous overgrowth, vascular malformations,
epidermal nevi, and skeletal/ scoliosis and spinal abnormalities [Sapp et al., 2007; Alomari,
2009]. The MCAP syndrome was characterized by a core set of brain features including
megalencephaly, ventriculomegaly that may progress to hydrocephalus, cerebellar tonsillar
ectopia that may progress to Chiari malformation, and cortical brain abnormalities
(specifically polymicrogyria or PMG). There are similarities in clinical findings to those
seen in MPPH. The MCAP syndrome can be distinguished from MPPH syndrome based on
somatic features typically recognized at birth, such as cutaneous vascular malformations,
especially capillary malformations of the face and cutis marmorata; cutaneous syndactyly
and postaxial polydactyly or polydactyly; connective tissue dysplasia and focal or
segmental body overgrowth [Mirzaa et al., 2013; Riviere et al., 2012]. It is clear that while
there may be some unique features for each diagnosis, there exists considerable overlap
between PROS. In addition, with further PIK3CA testing, the spectrum may widen to
include other distinct but related phenotypes, such as Klippel-Trenaunay Syndrome (KTS)
[Kurek et al., 2012].
DIFFERENTIAL DIAGNOSIS

Various syndromes considered in the differential diagnosis of PROS have overlapping clinical findings, such as hemihyperplasia, overgrowth, vascular anomalies, skin abnormalities, tumors, scoliosis, and others. However, these other overgrowth syndromes have similar, but more distinguishing features and other identifiable genetic etiologies. They include Proteus syndrome (PS) [Biesecker et al, 1999; Lindhurst et al., 2011], PTEN Hamartoma Tumor Syndrome (PHTS) and Type II segmental Cowden syndrome [Nelen et al., 1996; Eng, 2000; Eng 2001], Neurofibromatosis, type 1 (NF1) [Friedman JM, 1998; Ferner et al., 2007; Williams et al., 2009], and Epidermal Nevus syndrome [Laura, 2013].

For several of the participating research groups, the original referring diagnosis for many of their patients was described as PS, NF1, and KTS; however, the patients' findings did not meet published diagnostic criteria for these disorders. In particular, for example, none of the patients had connective tissue nevi characteristic of Proteus syndrome. The KTS includes overgrowth and vascular malformations, which are seen in the PIK3CA-Related Overgrowth Spectrum, and there is some debate about the extent of the diagnostic clinical findings. Indeed, PIK3CA mutations were identified in some individuals with a KTS-like phenotype [Kurek et al., 2012].

TESTING ELIGIBILITY CRITERIA

We have developed guidelines that clinicians can use in deciding which patients with overgrowth should be tested for a PIK3CA mutation. This determination is non-trivial as testing generally requires a biopsy. An exception to this is the testing for MCAP, which may be diagnosed by testing of a blood or saliva sample. Testing eligibility criteria (Table III) are based upon the clinical criteria described in Table II, but were less strict to include a wider range of conditions. While it is important to recognize and describe preexisting disease entities, key criteria were described to prompt clinicians to obtain testing for non-descript and potentially as-yet undiagnosed syndromes with features described for PROS. Accompanying these diagnostic features, functional abnormalities may also prompt consideration of testing. These may include genitourinary abnormalities such as urinary incontinence, hydronephrosis, hydroureter, gastrointestinal abnormalities such as constipation, gastrointestinal bleeding, and neurologic abnormalities including, seizures, autism, and intellectual disability.

Diagnostic genetic testing for the PROS poses several challenges. A key challenge is to determine which tissue has the greatest likelihood of having a detectable mutation. This is related to technical challenges with genetic testing, particular the sensitivity and specificity of available genetic testing technique(s). Biopsies from some PROS patients had low levels of mosaicism (<5%). Determining the optimal tissue for biopsy is sometimes difficult because it depends on gross visual assessment of overgrown or affected regions. In considering the diagnosis of mosaic disorders in general, detection of the causative mutation depends not only on the particular effects of the mutation (degree of overgrowth), but also on its load and distribution in the tissue. Identification of a low-level somatic mutation may therefore require new analytic methods.
In the experience of the workshop participants using current techniques, molecular genetic testing for the diagnosis of PROS requires clinically affected tissue samples, preferably freshly obtained from dermal biopsy overlying an affected area or from a surgical procedure of the overgrown tissue. This is because *PIK3CA* mutations can be detected in affected tissues or cultured cells at greatly varying levels [Keppler-Noreuil et al., 2014]. We agreed that testing of blood or DNA isolated from blood is not recommended based upon current techniques, as *PIK3CA* mutations have not been identified in the blood by any of the laboratories of the participating groups, except in 2 of 24 patients with MCAP, who had an apparent *de novo* germline mutation in *PIK3CA* [Riviere et al., 2012]. Testing can be performed using formalin-fixed paraffin-embedded (FFPE) tissue, but with varying degrees of success, and there is a need to ensure rigorous handling of the samples because of risk of contamination from the microtome. *PIK3CA* mutation detection in other tissues or fluids is currently being evaluated on a research basis including saliva, urine, lymph fluid, hair, and skin scrapings. Mutation analysis of saliva samples has not detected any mutations, except in many patients with MCAP and one reported patient with overgrowth of the salivary glands (unpublished data).

The types of assays and their respective sensitivities were described and are shown in Table IV. The choice of method for somatic mutation detection in *PIK3CA* depends on several factors. Several manufacturers have developed PCR-based assays for detection of specific *PIK3CA* mutations commonly found in cancers, and many of these mutations have also been found in patients with PROS as well. The data to date suggest that while there are a number of mutational hotspots (e.g., *PIK3CA* codons 542, 545, and 1047) there are a substantial number of rare mutations (Figure 2). The hot spot mutations have significantly elevated biological and biochemical activities (gain-of-function) above the rare mutations; these mutants may induce higher numbers of transformed cell foci suggesting more rapid cell proliferation or greater oncogenic potency. The other mutations may be grouped by oncogenic potency into strong, intermediate and weak [Gymnopoulos et al., 2007]. The use of custom RFLP assays or digital droplet PCR are typically mutation-specific and pose a tradeoff of ease of use versus breadth of the test. Other assays are available for screening multiple sites with a single reaction either by pooling primer pairs or by using a multi-well format. These tests sacrifice sensitivity for the ability to assay multiple sites. In order to detect new or very rare mutations, sequencing entire exons is necessary. Sanger sequencing can be used for this only if the mutation level is relatively high (~20%). Lower levels of mosaicism can be detected using this method, but usually only at positions where multiple patients have varying levels of the mutation and sequence traces can be extensively compared to distinguish background peaks from low levels of a variant. Targeted capture of the entire *PIK3CA* coding region followed by next generation sequencing at very deep coverage may be better suited for somatic mutation detection, as it allows for detection of very low levels of mosaicism throughout the gene. Optimization of these assays is an active area of research.

It is important to interpret the result based upon the particular assay and the tissue sampled. The finding of a mosaic mutation can be very useful to establish a diagnosis of a PROS disorder; however, there is a poor correlation of the mutation level (in either tissues or
cultured cells) to either the quality (nature) of the manifestations or the overall severity of the manifestations in the patient. Importantly, failure to detect a PIK3CA mutation does not necessarily exclude a clinical diagnosis of PROS in individuals meeting the clinical criteria described earlier, as this may be due to limitations in detecting mosaicism from sub-optimal tissue biopsies. We hypothesize that the overall lack of correlation of severity to mutation burden or lack of a genetic diagnosis emanates from the clinical sampling constraints. The ability to sample appropriate tissues is limited both by patient considerations and surgical constraints.

**FUTURE DIRECTIONS**

We discussed the importance of developing a collaborative and uniform phenotyping and clinical evaluation approach with the objectives of 1) compiling data on natural history and the extent of the associated complications of PROS, and 2) to provide useful outcome measures for use in therapeutic drug trials. The results of these evaluations may form the basis for assisting clinicians’ management of these patients, providing recommendations for surveillance for complications of these disorders. There have been reviews of the more commonly associated complications of this group of disorders, which forms a basis for the recommendations of clinical imaging [Alomari et al., 2010; Alomari et al., 2011; Kurek et al., 2012; Keppler-Noreuil et al., 2014]. We agreed that these evaluations (shown in Table V) should be based on the characteristic component clinical findings in the spectrum, including: somatic overgrowth (limb, trunk-spine, craniofacial), CNS overgrowth or dysplasia (brain and spine), vascular anomalies (in particular thrombosis), dermatologic, genitourinary, gastrointestinal, tumorigenesis, development, and endocrine/metabolism involvement. These 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 by Keppler-Noreuil et al. [2014]. Although the evidence is not sufficient to demonstrate high risk, it would be prudent to consider serial abdominal ultrasounds every 3–4 months until age 8 years in 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 vascular and lipomatous lesions involving the spine, neurological monitoring, and spinal MRI scan should be considered in patients with truncal involvement [Alomari et al., 2011; Keppler-Noreuil et al., 2014]. Finally, a reported risk of pulmonary embolism in patients with CLOVES syndrome having thoracic and central phlebectasia [Alomari et al., 2010] and in two separate patients with spinal thrombosis and neonatal cerebral infarcts [Keppler-Noreuil et al., 2014] 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.

Future longitudinal studies of larger cohorts are needed to further our understanding of the extent of the findings and natural history of PROS. A consortium or registry to aggregate clinical and molecular data may facilitate future studies of genotype-phenotype correlations.
and improve treatment recommendations. The family support groups expressed interest in participating and playing a role in the governance of this registry.

The workshop participants are at once gratified by the progress that has been made to date and humbled by the challenges that lie ahead. With newfound genetic understanding of PROS, sorting out diagnostic labels is relatively straightforward compared to the imperative to comprehensively understand the variable expressivity and natural history of patients with PROS. We are optimistic that the diagnostic criteria propose herein will reduce confusion among clinical professionals and patients and stimulate the research community to think broadly and comprehensively about these disorders. Most importantly, it is our primary objective to develop targeted effective treatments for patients with these disorders. We are extremely optimistic that this can be accomplished now that the door on the pathophysiology of these remarkable disorders has been opened.

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REFERENCES


Text Box 1

1. Aggregate clinical and molecular data from patients to summarize the spectrum and clinical delineation of PROS

2. Develop consensus on clinical diagnostic categories, proper nomenclature for the entities and potential molecular correlations

3. Propose an agenda for moving forward with considerations for both symptomatic and primary treatment.
Figure 1.
PI3K-AKT Pathway and associated clinical overgrowth disorders.
Figure 2.

PI3K-related overgrowth spectrum (PROS). PI3K predicted protein mutations and associated clinical overgrowth disorders discovered to date. Oncogenic potency: *Hot spot mutations, **Strong mutations, and +Intermediate [Gymnopoulos et al., 2007]
Figure 3.
Phenotypic Spectrum of PROS: disorders have overlapping clinical features, some with tissue-specific, localized effects, some with pleiotropic and more severe manifestations; FAO/HHML, Fibroadipose Overgrowth/Hemihyperplasia-Multiple Lipomatosis; ILM, Isolated Large Lymphatic Malformation; CLOVES, Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal; EN, Epidermal Nevi; SK, Seborrheic Keratoses; BLK, Benign Lichenoid Keratoses; MCAP, Megalencephaly-Capillary Malformation; HMEG, Hemimegalencephaly; DMEG, Dysplastic Megalencephaly
### TABLE I

#### Formal Presentations at the Workshop

<table>
<thead>
<tr>
<th>Topic</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>Biology of the PIK3CA pathway and clinical report of pilot therapeutics</td>
<td>Robert Semple</td>
</tr>
<tr>
<td>Clinical data on 24 patients with PIK3CA mutations from the NIH and Cambridge collaboration</td>
<td>Kim Keppler-Noreuil</td>
</tr>
<tr>
<td>CLOVES syndrome and PIK3CA mutations</td>
<td>Matthew Warman</td>
</tr>
<tr>
<td>Macrodactyly and PIK3CA mutations</td>
<td>Jonathan Rios</td>
</tr>
<tr>
<td>Asymmetric brain overgrowth in PIK3CA</td>
<td>William Dobyns</td>
</tr>
</tbody>
</table>
TABLE II

Clinical Diagnostic Criteria for PIK3CA-Related Overgrowth Spectrum (PROS)

| Required: Presence of somatic PIK3CA mutation* |
| Congenital or Early Childhood Onset |
| Overgrowth Sporadic and Mosaic (Other terms: Patchy, Irregular) |
| Features as described in either A or B |

A. Spectrum (two or more features)**
1. Overgrowth: Adipose, Muscle, Nerve, Skeletal
2. Vascular Malformations: Capillary, Venous, Arteriovenous Malformation, Lymphatic
3. Epidermal Nevus

B. Isolated features
1. Large Isolated Lymphatic Malformation
2. Isolated Macroductyly*** OR Overgrown Splayed Feet/ Hands, Overgrown Limbs
3. Truncal Adipose Overgrowth
4. Hemimegalencephaly (bilateral)/ Dysplastic Megalencephaly/ Focal Cortical Dysplasia ²
5. Epidermal nevus²
6. Seborrheic Keratoses²
7. Benign Lichenoid Keratoses³

Abbreviations: + present; − absent; HC hydrocephalus; ID intellectual disability

* If no mutation identified, then consider as presumptive PROS

** Typically Progressive. Can manifest as: Scoliosis (Kyphosis), Limb overgrowth, CNS (HC, Cerebellar tonsillar ectopia, Chiari, Megalencephaly, Mega corpus callosum, Regional lipomatous undergrowth with overgrowth, Infiltrating lipomatosis, Wilms tumor/ovarian cystadenoma

*** Other terms: macrodystrophia lipomatosa, macroductylia fibrolipomatosis and gigantism

¹ Dobyns WB, 2014 (unpublished data);
² Hafner et al. [2007];
³ Groesser et al. [2012]
**TABLE III**

Testing Eligibility Criteria for Somatic **PIK3CA** Mutations

**PIK3CA** mutation analysis should be performed, if a patient has one or more of:

**Key Features:**
- Congenital Spectrum (A)* or Congenital Stand Alone (B) *
- Combined Vascular Malformation – Large Capillary, Lymphatic, or Venous malformation
- Congenital Musculoskeletal Overgrowth
- Pattern defect** (e.g., Polydactyly, Sandal gap, Syndactyly)
- Congenital CNS (PMG/ MEG/ HC/ Chiari/ Syrinx)
- Congenital Epidermal Nevus (EN)
- Congenital soft doughy skin or joint hypermobility

*+/− Functional features: Hydronephrosis/ Hydroureter, Urinary Incontinence, Hematuria, Constipation, Gastrointestinal Bleeding, Intractable Epilepsy, Seizures, Intellectual Disability, Autism, Hypoglycemia

Abbreviations: CNS Central Nervous System; HC hydrocephalus; MEG megalencephaly; PMG polymicrogyria.

* Refer to these specific findings in Table II

** Upper (UE) and Lower Extremity (LE) findings may include, UE: broad spade-like hands with ulnar deviation of the digits, symmetrical overgrowth of 1 or more digits that does not usually follow a nerve territory-oriented pattern, laxity of collateral ligaments, furrowed palms and soles. LE: Overgrowth of the feet, which presents as a large “sandal” gap between great and second toes, large bulbous toes, lipomatous masses on both dorsal and plantar surfaces, broad forefoot with wide gaps between the metatarsal heads; dislocated knees, leg length discrepancy, and patellar chondromalacia [Bloom and Upton, 2013]
### TABLE IV

Current Assays for Testing of Somatic PIK3CA Mutations

<table>
<thead>
<tr>
<th>Method</th>
<th>Instrumentation needed</th>
<th>Maximum number of mutations assayed per test</th>
<th>Mutation specific</th>
<th>Detection Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>custom RFLP</td>
<td>fluorescent sequencer</td>
<td>1</td>
<td>+</td>
<td>0.5-1%</td>
</tr>
<tr>
<td>digital droplet PCR</td>
<td>digital PCR system</td>
<td>1</td>
<td>+</td>
<td>0.01-0.001%</td>
</tr>
<tr>
<td>snapshot assay</td>
<td>fluorescent sequencer</td>
<td>10</td>
<td>+</td>
<td>5-10%</td>
</tr>
<tr>
<td>qBiomarker array</td>
<td>qPCR instrument</td>
<td>93</td>
<td>+</td>
<td>1%</td>
</tr>
<tr>
<td>MassARRAY system</td>
<td>MALDI-TOF mass</td>
<td>200</td>
<td>+</td>
<td>5%</td>
</tr>
<tr>
<td>Sanger sequencing</td>
<td>fluorescent sequencer</td>
<td>NA</td>
<td>–</td>
<td>5-20%</td>
</tr>
<tr>
<td>next generation sequencing</td>
<td>next gen sequencer</td>
<td>NA</td>
<td>–</td>
<td>1-10%*</td>
</tr>
</tbody>
</table>

*Level of detection is dependent on depth of coverage at the mutant site; the lower the level of mutation, the greater the read depth needs to be for detection.
TABLE V

Clinical Imaging Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body MRI scan</td>
<td>- if there is truncal overgrowth or involvement (spine curvature)</td>
</tr>
<tr>
<td>For infants 6-12 months</td>
<td>- earlier age if symptomatic: contrast-enhanced study is preferable</td>
</tr>
<tr>
<td>For older patient</td>
<td>- as a baseline study with contrast (contrast may not be required depending on experience)</td>
</tr>
<tr>
<td>Cranial MRI scan</td>
<td>- if there is facial or neurologic involvement</td>
</tr>
<tr>
<td>Plain radiographs of affected areas</td>
<td></td>
</tr>
<tr>
<td>Spine series</td>
<td>- if there is curve on exam</td>
</tr>
<tr>
<td>Spinal canal ultrasonography</td>
<td>- as baseline in neonates and young infants with truncal involvement for tethered cord and lipomeningomyelocele</td>
</tr>
<tr>
<td>Renal ultrasonography</td>
<td>- at baseline, then every 3 months until age 8 years</td>
</tr>
</tbody>
</table>