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ACMG PRACTICE RESOURCE

Isolated lateralized overgrowth and the need for tumor screening: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG)

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ABSTRACT

Purpose: To provide diagnostic guidance for individuals with lateralized overgrowth (LO) and implement appropriate screening protocols. LO without a syndromic presentation is considered idiopathic isolated lateralized overgrowth (ILO).

Methods: We performed a literature search of LO syndromes and malignancy risk and reviewed existing guidelines and expert input.

Results: We integrated 940 unique articles to form recommendations. We defined LO as significantly larger length and/or girth of aspect(s) of one side of the body compared with its contralateral side. It can be associated with somatic overgrowth syndromes. ILO was previously defined based on clinical features and deemed idiopathic by absence of molecular findings. Much of the tumor risk is likely because of specific LO syndromic causes now identified through improved diagnostic technologies; therefore, the tumor risk in idiopathic ILO is likely lower than previously accepted.

Conclusion: Mosaicism complicates molecular diagnosis for children with LO. However, conditions such as Beckwith-Wiedemann spectrum and *PTEN*-related hamartoma tumor syndrome necessitate routine tumor screening. Establishing a specific diagnosis via comprehensive molecular testing on affected tissue will guide screening and management. In cases of idiopathic ILO, location of the overgrowth, estimation of tumor risk, regional practice approaches and family concerns all play roles in determining tumor screening.

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Introduction

When 1 or more parts of the body measure significantly larger than expected when compared with normal growth parameters, the term "overgrowth" may be applied. It has long been observed that some individuals with overgrowth tend to develop other clinically important features, such as neurodevelopmental phenotypes, other congenital malformations, and, most notably, an increased chance of developing certain types of cancer. However, the precise risk of cancers varies widely among these conditions. Proper classification of the overgrowth conditions is imperative to providing an accurate assessment of tumor risk, so that patients more likely to benefit from increased surveillance can be distinguished from those who are not.

Advances in genetic and epigenetic testing and particularly improvements in the detection of low-level mosaic molecular alterations have led to improved classifications of conditions featuring overgrowth. A "dyadic approach," in which genetic conditions are classified by the combination of genotype (eg, "PTEN related") and the phenotype ("hamartoma syndrome") has been widely adopted.[1] Examples of well-defined overgrowth conditions described by this dyadic approach include 11p15.5 methylation-related Beckwith-Wiedemann syndrome (BWS), 11p15.5 deletion-related BWS, *CDKN1C*-related BWS, *PIK3CA*-related overgrowth spectrum (PROS), *AKT1*-related Proteus syndrome (PS), *AKT1*-related overgrowth spectrum, and *PTEN*-related hamartoma tumor syndrome (PHTS). All of these conditions have an increased tumor risk, although the exact risk is dependent on the specific genetic or epigenetic cause, and in some instances warrants periodic screening for neoplasms.[2-5]

Complicating overgrowth further is the fact that it can also be classified based on its distribution in the body. Overgrowth can be "generalized," affecting all of the body proportionally, but individuals with localized overgrowth, which we now refer to as "lateralized overgrowth" (LO), are much more common. Individuals with isolated LO (ILO) with no identifiable molecular cause are considered to have idiopathic isolated lateralized overgrowth (idiopathic ILO). Idiopathic ILO was previously referred to as "isolated hemihypertrophy" or "isolated hemihyperplasia."[6] However, these terms are now considered obsolete because they may not accurately reflect the pathologic process.[7] In 2009, ACMG practice guidelines recommended that all children diagnosed with isolated hemihypertrophy and isolated hemihyperplasia be screened for renal and hepatic malignancies until age 7, by which time >95% of tumors would have occurred. These recommendations were based on the assumption that children with idiopathic ILO have a 5% approximate lifetime risk of developing a Wilms tumor and/or hepatoblastoma. However, our knowledge of the molecular causes of the various overgrowth syndromes and the sensitivity of our testing for somatic variants have improved as has our understanding of the role mosaicism and tumor risk of the variants play in the pathogenesis of overgrowth syndromes. Examples include mosaic genetic and epigenetic causes of BWS and somatic mosaicism in the PI3K-AKT signaling pathway, any of which can lead to overgrowth and may only be detected by sensitive assays on affected tissue.[8-10] Recognizing individuals with specific overgrowth conditions due to mosaic variants or epigenetic differences is challenging because the phenotypes can be highly variable and mild. This creates a clinical diagnostic challenge because the clinical features can be subtle compared with individuals with classic phenotypes. Evaluation for mosaicism or somatic variants in affected tissue has historically not been part of the testing for LO and remains underutilized. It is therefore likely that in a significant number of individuals with ILO, a molecular cause—and therefore a diagnosis of a specific overgrowth syndrome—may have been missed.[11]

The complexity of overgrowth conditions and insufficient sensitivity of the genetic or epigenetic work up in the past likely led to an inflated estimate of cancer risk in individuals with "isolated hemihyperplasia." In this practice resource, we reevaluate the 2009 recommendations in light of the significant advancements that have taken place in the interim.

Several key items are addressed in this clinical practice resource:

- Delineating a specific definition of isolated lateralized overgrowth.
- Clarifying a diagnostic strategy to ensure that defined overgrowth syndromes are diagnosed whenever possible.
- Emphasizing the role of mosaicism and somatic variants and how they should be captured in the diagnostic evaluation.
- Providing recommendations for tumor screening in individuals with idiopathic ILO.

Although this document is focused on diagnosis and management of ILO, a brief overview of the most common and well-described overgrowth syndromes is included.

Materials and Methods

A literature review of the malignancy risk in ILO, as well as the most common syndromes characterized by lateralized overgrowth, was performed with the help of a biomedical librarian using Ovid Medline. The search spanned January 2008 to October 2021 and included only articles published in English. Search terms are listed in Supplemental Table 1. Because of a paucity of scientific evidence to facilitate diagnosis and management of idiopathic ILO, expert opinion was heavily relied upon in making these recommendations.

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Results

Literature review

Nine hundred and forty articles were identified that contained the search terms either in the title, abstract, original title, name of substance word, subject heading word, floating subheading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, or synonyms. These articles were further reviewed to determine if they contained data on the incidence or prevalence of malignancies in individuals who may have ILO to ensure that the most recent evidence was used to establish this clinical practice resource.

Definition and diagnosis of isolated lateralized overgrowth

In 2017, the terms "isolated hemihypertrophy" and "isolated hemihyperplasia" were replaced by LO, defined as "significantly larger length and/or girth of aspect(s) of one side of the body compared with its contralateral side."[12] Lateralized overgrowth can be restricted to one side of the body, but crossing of the midline may be present, which is why the term "lateralized" is preferred over "unilateral." It can be difficult to ascertain if the difference in size is caused by underdevelopment of the smaller side, overdevelopment of the larger side, or a combination of both. Generally, it is thought that overgrowth is more easily appreciated than underdevelopment.[6]

The diagnosis of LO is usually based on physical examination. Although it is assumed that length or girth discrepancies of 10% or more are usually visually apparent, experienced examiners may be able to detect more subtle asymmetries. Given the broad variability in individuals with LO and the subjective nature of physical examinations, no objective parameters have been established to determine at which point the degree of asymmetry should be considered nonphysiologic. It is generally left to the examiner to decide if the findings are significant enough to warrant further evaluation.

Several aspects need to be considered in the assessment and physical examination of an individual with overgrowth that help to determine the most appropriate diagnostic path and clinical management (Figure 1). The most important factors that play a role in determining which category of conditions the overgrowth falls into include extent and distribution of overgrowth, proportionality, and timeline.

Extent and distribution of overgrowth

- Generalized overgrowth: if overgrowth affects both sides of the body or cranial/facial structures are involved, conditions such as Sotos syndrome, BWS,

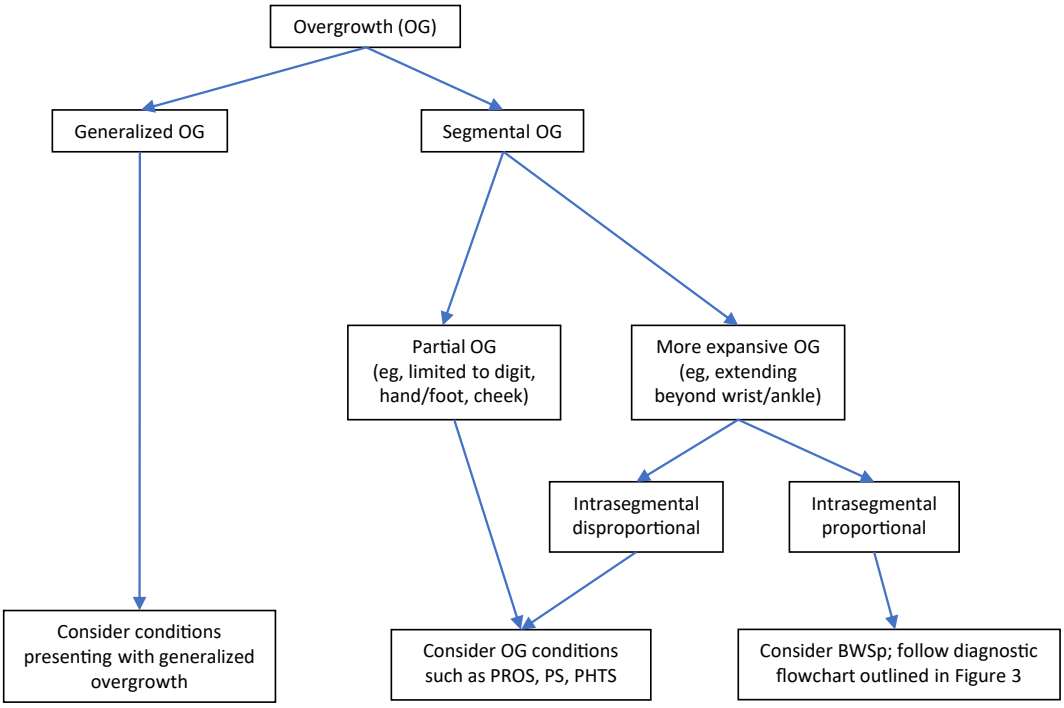

Figure 1 Characterization of overgrowth pattern based on physical examination findings. Based on the clinical presentation, there may be a suspicion for a certain disease group and testing for those conditions should be prioritized. Affected individuals with ILO will fall under the category "intrasegmental proportional overgrowth," and the most appropriate diagnostic pathway for this group of affected individuals is outlined in Figure 3. BWSp, Beckwith-Wiedemann spectrum; ILO, isolated lateralized overgrowth; PHTS, *PTEN*-related hamartoma tumor syndrome; PROS, *PIK3CA*-related overgrowth spectrum; PS, *AKT1*-related Proteus syndrome.

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PHTS, and other overgrowth syndromes affecting the entire body should be considered.

- Segmental overgrowth: usually lateralized but crossing of the midline can be present. The extent of the overgrown segment can range from focused, subtle segments to widespread profound growth, including complete LO of an entire limb:
  - Focal overgrowth (eg, cheek, digit, hand, or foot without extension beyond wrist or ankle) often implicates conditions such as PROS/PS.

Proportionality

If segmental overgrowth is present, proportionality of the growth can be helpful in assessing underlying etiology. Intrasegmental vs intersegmental proportionality should be distinguished.

Definition of proportionality terms:

- Intrasegmental proportionality: the overgrown segment is proportionate in itself. For example, all fingers of an overgrown hand are proportionate when compared with each other. Or from a clinical perspective, when viewed in isolation, the single hand appears normal. The overgrowth is only appreciated when comparing with the contralateral or adjacent segments.
- Intrasegmental disproportionality: the overgrown body part is disproportionate in itself. For example, the fingers of an overgrown hand are different in size when compared with each other. Accordingly, when viewing the hand in isolation, the variability in finger growth is appreciable.
- Intersegmental disproportionality: the overgrown body part is larger than adjacent body parts. For example, hand length or bulk is notably larger compared with the forearm or upper arm.

When applied to affected individuals, 2 main clinical presentations for LO tend to predominate:

- Intrasegmental proportionality with intersegmental disproportionality (Figure 2A-D): the overgrown part is proportionate within the segment but disproportionally large in comparison with contralateral or adjacent body structures/parts. An example includes an overgrown foot and lower leg, in which the foot including toes is proportionate in itself and in comparison with the lower leg but disproportionately larger in relation to the upper part of the leg and contralateral leg. This pattern is suggestive of Beckwith-Wiedemann spectrum (BWSp) or chromosome 11p-mediated mosaicism.
- Intrasegmental disproportionality (Figure 2E): the overgrown body part is disproportionate within the segment. An example includes an overgrown hand with differently sized fingers (with respect to length, as well as circumference). Intrasegmental disproportionality is more often associated with PROS and when the disproportionality is also "distorting," it is more suggestive of PS.[13]

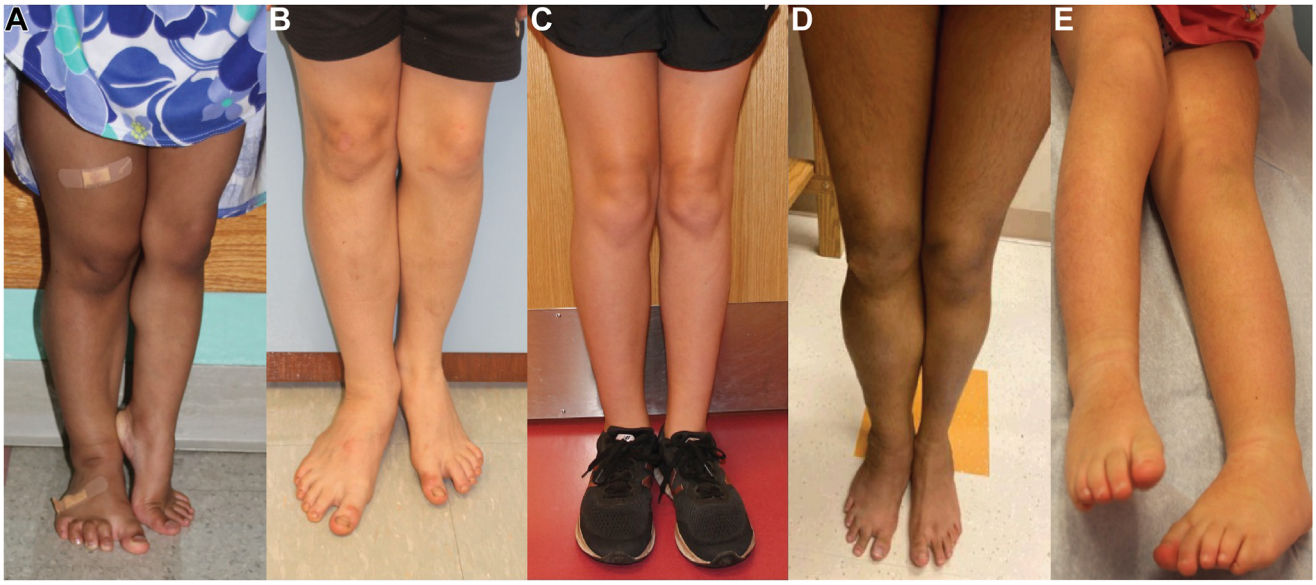

Figure 2 Examples of different types of segmental overgrowth. A-C. Segmental overgrowth of the lower extremity with intrasegmental proportionality but intersegmental disproportionality of differing degrees—affected individuals with BWSp. D. Segmental overgrowth of the lower extremity with intrasegmental proportionality but disproportionality in comparison with the adjacent unaffected body parts—affected individual with PHTS. E. Segmental overgrowth of a foot and leg with intrasegmental disproportionality and undistorted appearance in an individual with PROS. BWSp, Beckwith-Wiedemann spectrum; PHTS, *PTEN*-related hamartoma tumor syndrome; PROS, *PIK3CA*-related overgrowth spectrum.

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Timeline and progression

Additional important factors when assessing LO are timeline and progression.

- LO noted at or shortly after birth (in infancy) suggests BWSp, particularly if additional examination findings are consistent with this diagnosis (see below). However, subtle asymmetry may not be appreciated at birth. In our experience, most clinically relevant asymmetry in BWSp can be noted by 6 to 12 months. LO in BWSp tends to be relatively stable through childhood with respect to the percent difference between sides and then tends to be less apparent in late childhood and adolescence.
- LO associated with PROS is typically present at birth but can demonstrate more rapid progression than that caused by BWS and can continue to progress into adulthood.
- LO caused by PS typically does not present until after 18 months to as late as 12 years and is often rapidly progressive.

When evaluating individuals presenting with overgrowth, using the diagnostic algorithm in Figure 1 may be helpful when determining the specific overgrowth pattern and deciding which diagnostic path to pursue. Phenotypic findings may strongly point toward conditions such as PS, PROS, or PHTS, in which case disease-specific testing is most appropriate. Individuals who fall into the category of "intrasegmental proportional overgrowth" may have subtle presentations, and there may not always be a high suspicion for a specific disorder. In those cases, BWSp is usually highest on the list of differential diagnoses, and a diagnostic workup as outlined in Figure 3 is recommended.

Differential diagnosis of lateralized overgrowth

The most well-described overgrowth syndromes associated with LO have some degree of overlapping clinical features that can complicate the diagnostic process of an individual presenting with LO; however, distinct clinical features, if present, can guide the diagnostic workup. The clinical presentations, molecular testing nuances, and the tumor risk and screening implications of these conditions are described below. More rare overgrowth conditions that are less common or less well described than those below should be included on somatic overgrowth panels ordered for those affected individuals presenting with LO who undergo a tissue biopsy for molecular testing.

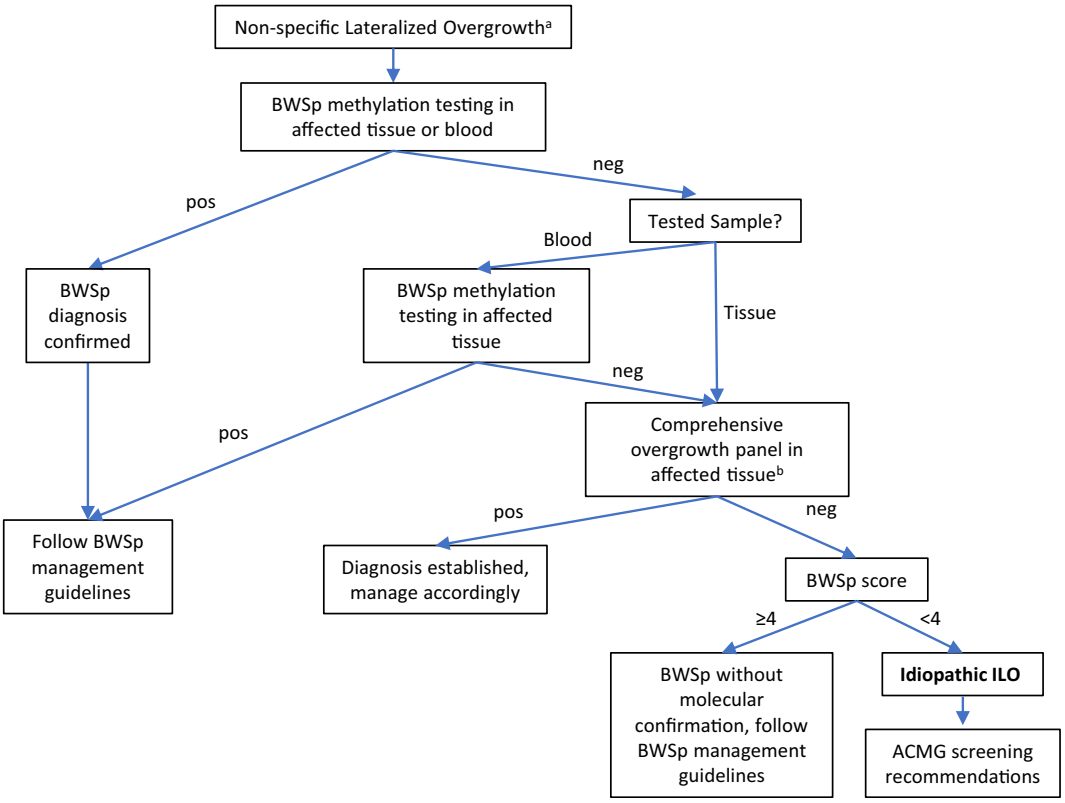

Figure 3 Diagnostic flowchart for individuals with lateralized overgrowth. [a]Isolated lateralized overgrowth = lateralized overgrowth that does not meet criteria for a specific defined overgrowth condition. If the phenotype points toward a specific overgrowth condition, disease-specific testing may be a more appropriate first test. [b]Comprehensive overgrowth panels should include at a minimum BWSp, PS, PROS, PHTS, and NF1. BWSp, Beckwith-Wiedemann spectrum; neg, negative; NF1, *NF1*-related neurofibromatosis 1; PHTS, *PTEN*-related hamartoma tumor syndrome; pos, positive; PROS, *PIK3CA*-related overgrowth spectrum; PS, *AKT1*-related Proteus syndrome.

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BWSp

BWSp, which includes classic BWS, is the most common cancer predisposition and overgrowth syndrome.[14] It is characterized by the cardinal features of LO, macroglossia, omphalocele, hyperinsulinism, bilateral Wilms tumor, and certain pathological anomalies, including placental mesenchymal dysplasia and adrenal cytomegaly. Suggestive features of BWS include facial nevus simplex, ear creases or pits, transient neonatal hypoglycemia, placentomegaly or polyhydramnios, umbilical hernia or diastasis recti, nephromegaly, hepatomegaly, and specific embryonal tumors.[15] Because of the range in number and severity of features seen across individuals with BWS, the condition was reclassified as BWSp by an international consensus group.[15]

A diagnosis of BWSp can be made through a clinical scoring system or through molecular testing. To establish a clinical diagnosis of BWSp, a score of 4 points or greater is required, with cardinal features of BWSp being 2 points and suggestive features being 1 point.[15] Molecular testing is recommended for individuals with at least 2 points. Recommended testing includes methylation analysis of imprinting control regions 1 and 2 (IC1 and IC2) of chromosome 11p15 with copy-number variation analysis if positive. If methylation analysis is negative, sequencing of *CDKN1C* (HGNC:1786) is warranted, and repeat methylation in a second tissue should be considered because of mosaicism.[15,16] These methods detect the epigenetic and genetic causes of BWSp, which include loss of methylation at IC2 (50% of affected individuals), paternal uniparental isodisomy of chromosome 11p15 (20% of affected individuals), gain of methylation at IC1 (5% of affected individuals), pathogenic variants in *CDKN1C* (5% of affected individuals), and chromosomal differences including duplications, deletions, and translocations involving 11p15 (<5% of affected individuals).[17]

The overall risk for tumors in BWSp is 8%.[18] Previous cohort studies including children with milder phenotypic variants of BWSp suggested that risk could be stratified based on (epi)genotype; however, recent reports of discordant (epi)genotypes among tissues in the same affected individual suggest that further research on risk stratification including affected individuals representing the full spectrum of BWSp is needed.[19,20] The most common tumors seen in BWSp are Wilms tumor (52%) and hepatoblastoma (14%) with neuroblastoma, rhabdomyosarcoma, adrenocortical carcinoma, and pheochromocytoma occurring more rarely.[21] The recommended tumor screening protocol for affected individuals with BWSp is used for individuals with ILO without an identified molecular cause. Screening includes a complete abdominal ultrasound and monitoring alpha-fetoprotein (AFP) levels every 3 months until the third birthday followed by renal ultrasounds every 3 months from the 3rd through the 7th birthday.[12,22]

PTEN-related Hamartoma tumor syndrome

PHTS is a spectrum that encompasses multiple distinct phenotypes, including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), macrocephaly/autism syndrome, and nonspecific lateralized overgrowth.[23] A rare but distinct allelic entity of *PTEN*-related segmental overgrowth, lipomatosis, arteriovenous malformation, and epidermal nevus syndrome (also known as type 2 segmental Cowden syndrome) is more severe and is associated with biallelic *PTEN* (HGNC:9588) variants, 1 germline and 1 mosaic.[24,25] *PTEN*-related CS is a multisystem disorder that is characterized by macrocephaly, multiple hamartomas, and variable other features with increased risk of benign and malignant tumors in various organs including but not limited to skin, thyroid, breast, endometrium, and colon.[26] *PTEN*-related BRRS is associated with pigmented macules on the penis, intestinal hamartomas, lipomas, vascular anomalies, and macrocephaly. LO has been previously described.[27] It is important to note that there is broad variability of the clinical presentation in PHTS phenotypes and significant clinical and genetic overlap between the different conditions with some reported families having both CS and BRRS phenotypes in a single family with the same *PTEN* variant.[26,28,29] A pathogenic germline variant in *PTEN* was found in 30% to 35% of individuals meeting CS criteria and about 60% of individuals meeting BRRS criteria.[28,30]

The cancer risk varies among the various phenotypes within PHTS and has mostly been described in affected individuals with CS. Lifetime risk of female breast cancer has been found to be anywhere from 25% to 85% depending on the study.[31,32] Other cancer risks include lifetime epithelial thyroid cancer of 35%, kidney cancer of 33%, endometrial cancer of 28%, colorectal cancer of 9%, and melanoma of 6%.[32] The cancer risk of *PTEN*-related segmental overgrowth, lipomatosis, arteriovenous malformation, and epidermal nevus syndrome is unknown.

There are increased surveillance recommendations for individuals with *PTEN*-related PHTS because of the associated increased estimated cancer risk. Per National Comprehensive Cancer Network guidelines, it is recommended that females start breast cancer surveillance at age 30 with annual mammograms and consideration of breast magnetic resonance imaging with contrast. For thyroid cancer surveillance, annual thyroid ultrasounds starting at age 7 are recommended. For colon cancer risk, it is recommended to start surveillance colonoscopies at age 35 and then repeat every 5 years. Monitoring for kidney cancers starts at age 40 with annual renal ultrasound every 1 to 2 years. Given the risk for endometrial cancer, endometrial biopsies every 1 to 2 years starting at age 35 may be considered with a consideration of hysterectomy upon completion of childbearing.[33]

Proteus syndrome

AKT1-related PS is rare and typically presents with mild to moderate overgrowth, although severe congenital cases have been described.[4] In most cases, there is severe, rapidly progressive overgrowth beginning at 1 to 2 years of age. This rapid overgrowth may affect any tissue but most commonly involves skeletal and skin overgrowth, including the characteristic cerebriform connective tissue nevus.

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Morbidity can be severe, and nearly 25% of affected individuals succumb by the age of 21.[34]

PS has a wide range of neoplasms, including meningiomas, genitourinary tumors, and breast tumors.[35] Most neoplasms associated with PS are benign, but some malignancies have been seen. It can be challenging to distinguish benign from malignant tumors in this disorder because some can have histologic features of both (C Ours, unpublished data).

PS is only associated with mosaic, activating *AKT1* (HGNC:391) variants, nearly always the NC_000014.9:g.104780214C>T c.49G>A p.(Glu17Lys) variant but occasionally the NC_000014.9:g.104780213_104780214delinsCT NM_001382430.1:c.49_50delinsAG p.(Glu17Arg) variant.[36] The molecular diagnosis requires testing of affected solid tissues because peripheral blood is uniformly negative.

Because of the rarity of the disorder and the wide range of tumors associated with PS, it has been difficult to develop robust data on the magnitude of the risk. Additionally, the heterogeneity of the observed tumors makes it difficult to develop specific tumor surveillance recommendations. Regular monitoring by clinicians caring for affected individuals is recommended at least annually for signs and symptoms of tumors. Expedited evaluations should be performed if concerning signs or symptoms arise.[4]

PIK3CA-related overgrowth spectrum

PROS describes a range of phenotypes characterized by the segmental overgrowth of 1 or more tissues including muscle, nerve, adipose, vascular, and brain. Vascular and lymphatic malformations, skin findings (eg, epidermal nevi and hyperpigmented macules), digital anomalies (eg, polydactyly and macrodactyly), renal anomalies, and differences on brain imaging (eg, hemimegalencephaly and cortical dysplasia) are also associated with this condition.[37] Previously described syndromes that are now considered part of PROS because of their common underlying genetic etiology include megalencephaly-capillary malformation syndrome, Klippel-Trenaunay syndrome, fibroadipose hyperplasia, and congenital lipomatous asymmetric overgrowth of the trunk, lymphatic, capillary, venous, and combined-type vascular malformations, epidermal nevi, skeletal, and spinal anomalies syndrome (CLOVES) among others.[38,39]

A diagnosis of PROS is confirmed via the identification of a pathogenic, activating variant in *PIK3CA* (HGNC:8975). Because most individuals with PROS have postzygotic, somatic variants in *PIK3CA*, molecular testing is complicated by mosaicism. Testing of an apparently affected tissue increases the chances of determining a molecular diagnosis, and the use of next-generation sequencing (NGS) with deep coverage is preferred for detecting a low-level variant.[40]

The most common benign tumor in individuals with PROS is a lipoma. Although there are reports of individuals with PROS having "hemangioma," these actually refer to capillary malformations, not vascular tumors. Two individuals with molecularly confirmed PROS have been reported with spinal neurofibromas (only 1 of which was confirmed histologically).[37] A small number of malignancies that have not been seen with enough frequency to consider surveillance have been reported in affected individuals with PROS. These malignancies include leukemia, retinoblastoma, rhabdomyosarcoma, astrocytoma, juvenile granulosa cell tumor, and borderline serous tumor.[3,41,42] The primary malignant tumor reported in PROS is Wilms tumor. In a recent meta-analysis, 6 affected individuals with PROS out of 483 affected individuals were reported to have Wilms tumor or nephrogenic rests. Of note, 5 of those individuals were diagnosed with CLOVES.[3] At this time, the data are insufficient to recommend standard Wilms tumor screening via quarterly abdominal imaging for affected individuals with PROS; however, a shared decision-making model for surveillance with families of affected individuals with a CLOVES phenotype can be considered.[22] It is currently not entirely understood why individuals with PROS do not have higher incidences of neoplasia.

Neurofibromatosis 1

Classical *NF1*-related neurofibromatosis 1 (NF1) presents with multiple café au lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, iris Lisch nodules, and plexiform neurofibromas. Cutaneous neurofibromas develop during adolescence and adulthood and about 10% of individuals develop malignant peripheral nerve sheath tumors.[43] The risk of breast cancer is also increased in women with this condition.[44]

Café au lait spots, typically ovoid in shape with well-defined borders, uniform in color and about 1 to 3 cm in size, are often present at birth and increase in number during the first few years of life. Freckling can be present in unusual, non-sun-exposed areas, including axilla and inguinal regions where skin rubs against skin. Congenital anterolateral tibial bowing is typically present at birth but can become more apparent upon ambulation. Diffuse plexiform neurofibromas of the face and neck rarely appear after the first birthday. Those on other parts of the body rarely develop after adolescence and typically remain stable throughout adulthood.[45] Deep nodular plexiform neurofibromas may be seen at any age; however, these are usually not symptomatic in childhood and often remain asymptomatic in adulthood. Benign cutaneous and subcutaneous neurofibromas are rare before late childhood and usually present in adults. Optic nerve gliomas in *NF1*-related neurofibromatosis are usually asymptomatic, and the majority of them can regress spontaneously.[46] Most individuals with *NF1*-related neurofibromatosis have normal intelligence; however, learning disabilities or behavioral problems occur in 50% to 80% of patients, intellectual disability in 6% to 7%, and autism spectrum in up to 30%.[47,48]

Mosaic or segmental *NF1*-related neurofibromatosis occasionally presents in the context of LO of an arm or leg in a child. This limb asymmetry is typically subtle, and

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key findings may include café au lait spots, congenital anterolateral tibial bowing of the larger side, or axillary or inguinal freckling.

Because of the nonclassical clinical presentation of the mosaic neurofibromatosis phenotype, genetic testing is warranted. Optimal testing would use an affected tissue, most often skin via a biopsy. Molecular testing would include sequence and deletion/duplication testing capable of detecting mosaic variants, typically NGS-based testing. Genes tested should include *NF1* (HGNC:7765) and, in individuals with atypical phenotypic presentation, genes related to overlapping conditions, including *SPRED1*-related Legius syndrome, *NF2*-related schwannomatosis,[49] RAS pathway genes associated with rasopathy phenotypes (*BRAF* [HGNC:1097], *KRAS* [HGNC:6507], *MAP2K1* [HGNC:6840], *NRAS* [HGNC:7989], *PTPN11* [HGNC:9644], *RAF1* [HGNC:9829], *RIT1* [HGNC:10023], and *SOS1* [HGNC:11187]), and *GNAS*-related fibrous dysplasia/McCune-Albright syndrome. Additionally, the clinician could consider constitutional mismatch repair deficiency and/or Bloom syndrome associated (*RECQL3* [HGNC:1058]) genes.

Malignant peripheral nerve sheath tumors, the most frequent malignant neoplasm in *NF1*-related neurofibromatosis, occurring in approximately 10% of affected individuals, can present with pain, neurologic deficit, or enlargement of a preexisting plexiform neurofibroma. These require immediate evaluation typically with examination by magnetic resonance imaging, positron emission tomography, or positron emission tomography/computed tomography.[43,50]

Health supervision for both classical and mosaic *NF1*-related neurofibromatosis would include monitoring for pain, development of a neurologic deficit, or enlargement of a preexisting plexiform neurofibroma prompting immediate evaluation for a malignant peripheral nerve sheath tumor.

Clinical diagnostic and management recommendations for isolated lateralized overgrowth

Evaluation of affected individuals with clinically undiagnosed or "nonspecific" LO (to be distinguished from affected individuals who have undergone thorough molecular evaluation with no etiology identified who have "idiopathic/undiagnosed" ILO).

Given the higher incidence and associated tumor risk, molecular analysis for BWSp should be the first step for all individuals with ILO, especially when there is intrasegmental proportionality. If there are features such as intrasegmental disproportionality associated with an overlying vascular malformation that are less consistent with BWSp, a broader overgrowth panel containing the *PIK3CA*,

Table 1 Physical examination findings in individuals with lateralized overgrowth

Overgrowth Syndrome	Typical Phenotypic and Clinical Findings	Limb-Specific Overgrowth Presentation
BWSp	Macroglossia, ear creases, umbilical hernia, neonatal hypoglycemia, and renal asymmetry (on US)	Intrasegmental proportionality. Major part, or full, limb. Noted at or shortly after birth. Not progressive.
PIK3CA-related overgrowth spectrum	Macrocephaly, vascular and lymphatic malformations, skin findings, skeletal anomalies, renal differences, and brain findings	Intrasegmental and intersegmental disproportionality. Can be limited to small areas. Can be associated with skin findings or vascular anomalies. Progressive.
PTEN-related hamartoma tumor syndrome	Macrocephaly, skin findings, autism, and vascular malformations	Intrasegmental and intersegmental disproportionality. Can be limited to small areas. Can be associated with skin findings or vascular anomalies. Progressive.
AKT1-related Proteus syndrome	Cerebriform connective tissue nevi, skin findings, lipomatous overgrowth and atrophy, and dysmorphic facial features	Intrasegmental and intersegmental disproportionality. Can be limited to small areas. Progressive.
NF1-related neurofibromatosis	Café au lait spots, axillary and inguinal freckling, neurofibromas, macrocephaly, and coarse facial features	Subtle. Can be associated with café au lait spots, freckling, and tibial bowing.

BWSp, Beckwith-Wiedemann spectrum, which is associated with several molecular etiologies (see text); *US*, ultrasound.

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PTEN, *AKT1*, and other overgrowth genes should be considered. The presence of distortion can be used as a distinguishing feature between PS and other conditions such as PROS and PHTS.

Many individuals do not fall neatly into a clear phenotypic category, especially very young individuals because certain overgrowth patterns may evolve over time. In the absence of a clear molecular diagnosis, we recommend following individuals longitudinally. Tumor screening may be pursued during this time on a case-by-case basis. In these individuals with nonspecific LO, BWSp has been shown to ultimately be the most common cause when a molecular diagnosis is ascertained; however, PROS has been identified in some affected individuals with nonspecific LO as well.[10] Thus, the most cautious approach is to follow the tumor screening guidelines for BWSp until a molecular or clinical diagnosis of a condition with lower tumor risk is made.[22]

Diagnostic pathway for LO

Along with a thorough clinical evaluation, BWSp methylation testing should be the first consideration in the diagnostic workup of an individual with LO (Figure 3). If the phenotypic presentation is more consistent with another overgrowth condition, such as PS, PHTS, PROS, or others (Table 1), a diagnostic approach targeting that disorder may be more appropriate as a first step. If initial blood or saliva-based testing for these conditions is normal and the clinical suspicion is sufficient, testing should be performed in a tissue affected by overgrowth. If all testing remains non-diagnostic, the individual's BWSp clinical score should be determined. A score of 4 points or above on the BWSp clinical scoring metric is consistent with a working diagnosis of BWSp without molecular confirmation, and further management should be informed by published guidelines.[15] If the BWSp clinical score is less than 4 points, a diagnosis of idiopathic ILO is established and management recommendations are outlined below.

Molecular diagnosis

Molecular testing is crucial for estimating recurrence risk and providing optimal patient care.[51,52] A causal finding has been reported in 80% of individuals with BWSp and 40% to 60% of affected individuals with non-BWSp LO.[16,53-55] Most LO syndromes are not inherited with a few notable exceptions, such as familial BWSp caused by *CDKN1C* pathogenic variants and certain chromosomal abnormalities.[56,57] Most individuals with non-BWS LO harbor somatic variants in genes involved in the PI3K-AKT-PTEN pathway and, less frequently, the RAS/MAPK pathway and other growth factor genes, such as *PDGFRB* (HGNC:8804).[53,58-60] Approximately 85% of individuals with BWSp and most individuals with other LO disorders are mosaic, meaning that the causal pathogenic alteration arose somatically and is not present in every cell in the body. The mosaic level of the alteration, known as variant allele frequency (VAF), can vary significantly among different tissues, posing challenges for molecular diagnosis. Testing multiple tissues, affected tissues, and in some cases, cultured tissues may be necessary to increase the diagnostic yield.[53,61] Highly sensitive methods, such as real-time quantitative or digital droplet polymerase chain reaction and ultra-deep NGS, are necessary for detecting very-low-level mosaic alterations.[16,62]

The molecular diagnostic strategy for BWSp is well established.[63] The first-line test is to determine the methylation status of IC1 and IC2 and the copy number of differentially methylated regions. The most commonly used technology is methylation-specific multiplex ligation-dependent probe amplification, which simultaneously detects the methylation status and copy number of differentially methylated regions.[64,65] However, because of limitations in sensitivity of methylation-specific multiplex ligation-dependent probe amplification, highly sensitive allele-specific methylated multiplex quantitative real-time polymerase chain reaction that allows detection of mosaic methylation changes and low-level copy-number alterations has been utilized in clinical laboratories.[16] If methylation testing is positive, SNV array analysis is warranted to characterize segmental vs whole chromosome uniparental disomy and cryptic chromosomal deletions/duplications.[15]

If methylation testing is negative, testing additional tissues should be considered as the next step. To avoid a potential methylation pattern change influenced by tissue culture, genetic testing of cultured cells is not recommended for this assay. Individuals with suspected BWSp (score $\geq$2), but negative for all above tests, should be further evaluated for alternative diagnoses with overlapping clinical features, such as Sotos or Simpson-Golabi-Behmel syndrome phenotypes.

Among individuals with non-BWS LO, activating somatic variants in the *PIK3CA* gene are the most common genetic alterations. Pathogenic *PIK3CA* variants are almost uniformly mosaic and present in affected tissues at VAFs as low as 1%. Thus, Sanger sequencing, with a typical limit of detection of over 20%, is not recommended. Genome- or exome-wide testing is also not recommended because the coverage obtained in these broad assays is usually insufficient to detect variants at this low level. Therefore, a comprehensive NGS panel using DNA derived from affected tissues is recommended after nondiagnostic BWS testing in individuals with nonspecific LO (Figure 3). For individuals with features suggestive of PROS or other non-BWS LO syndromes, a comprehensive NGS panel on affected tissue should be considered a first-tier test. A sensitive panel with limit of detection down to VAF $\geq$1% is recommended. The diagnostic yield of LO by sequencing a panel of genes is between 40% to 60%.[53,61] Some diagnostic labs prefer paired testing (eg, blood and affected tissues), because it easily resolves somatic from germline variants. However, it is generally easy for labs to distinguish low VAF somatic variants from germline variants even in the absence of a paired normal sample. We recommend

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nonpaired testing of affected tissue only to reduce complexity of the testing. In situations in which affected tissue is unexpectedly found to possess a variant at a VAF (>30%) possibly consistent with nonsomatic, germline variation, testing of another sample (blood, saliva, or parental sample) can then be used to clarify the state (mosaic or germline) of the variant.

Because of the complexity and cost of growing fibroblasts, and the possibilities of in vitro growth effects, we do not recommend culturing cells for diagnostic testing of LO. We recommend sequencing directly from frozen or formalin fixed paraffin embedded samples because this most accurately reflects the genetic state of the tissue being tested. However, in some cases, when the cell input is low (eg, amniotic fluid) or the lab is limited to less sensitive diagnostic testing (eg, Sanger), testing cultured cells may be considered.[53,66]

Tumor risk in isolated lateralized overgrowth

Idiopathic ILO has historically been associated with an increased risk for malignancy, most notably for Wilms tumor and hepatoblastoma. Studies before 2010 report a higher cancer risk than those from the past decade, reflecting an overestimation of tumor risk in older studies because of lower rates of comprehensive molecular testing and "lumping" of higher tumor risk conditions (eg, BWSp) with lower risk conditions (eg, PROS). In a landmark study by Hoyme et al[67] from 1998, observation of 168 individuals with ILO resulted in an estimated cancer risk of 5.4%. A smaller study with 40 affected participants revealed a tumor risk of 15%.[68] This assumed tumor risk of >5% prompted the 2009 ACMG recommendations for frequent tumor screening until age 7 because most (>95%) malignancies had been reported by this age.[7] To date, the largest group of individuals with ILO assessed for tumor risk was reported in 2012 by Dempsey et al.[69] In this retrospective review of 250 affected individuals over a 10-year period, the cancer prevalence was 1.2% (2 affected individuals developed Wilms tumor and one adrenal cancer).[69] Combining the Dempsey and Hoyme cohorts yields a tumor risk of 3.1% (13 tumors/418 individuals).[67,69] Atik et al[70] described a group of 24 individuals with ILO of whom 1 developed Wilms tumor, amounting to a cancer rate of 4.2%. Together, these findings indicate that the tumor risk is likely lower than previously thought.[11,67,69,71,72]

Medical management recommendations in isolated lateralized overgrowth

Although there is insufficient evidence to precisely estimate the cancer risk in individuals with ILO (negative comprehensive molecular evaluation and BWS score <4), the lifetime risk for malignancy is likely less than 5%. The question of whether this is sufficient to warrant tumor screening depends on the acceptable risk threshold of each individual and their family. Although a 5% tumor risk is viewed as acceptable in many European countries,[21,73,74] a risk threshold of 1% is more commonly applied in the United States.[12,75] Opponents of tumor screening in affected individuals with low tumor risk point to increased parental anxiety and the possibility of false positive results followed by unnecessary invasive testing.[76] Tumor screening in low-risk individuals increases costs to the health care system and can increase financial and logistical burdens to families. A recent US survey examining parental perspectives on tumor screening in BWS and ILO found that most parents viewed tumor screening as a means to decrease their worry regarding tumor development rather than experiencing it as a burden. This result included parents whose child had previously received a false positive screening result before the study.[77]

Our recommendations are that every effort should be made to identify a molecular etiology in individuals with LO to determine the most accurate tumor risk. Parents should be

Table 2 Recommendations for affected individuals diagnosed with LO

(1) Any child with a suspected LO condition should be referred to a clinical geneticist or medical provider familial with overgrowth conditions for evaluation.
(2) Diagnostic evaluation should be exhaustive and include testing of the affected tissue for overgrowth syndromes because establishing an underlying etiology may significantly change clinical management with respect to tumor surveillance, evaluation for other diagnosis-related symptoms, as well as counseling of the affected individual and/or the parents regarding recurrence risk.
(3) If, when following a thorough molecular evaluation, no molecular cause can be established in blood and affected tissue, and criteria for a clinical diagnosis of BWSp are not met, the individual can be diagnosed with idiopathic ILO.
    (a) Discuss the tumor risk with the parents (if comprehensive molecular work-up was performed including on tissue, tumor risk is likely <5%) and decide with the parents if tumor screening will be followed. In these children, a baseline abdominal ultrasound should be considered to assess for renal asymmetry:
        i. Symmetric kidneys (<1-cm difference in size): tumor risk can be assumed to be <1%, and ongoing screening may not be indicated.
        ii. Asymmetric kidneys (≥1-cm difference in size): ongoing screening for malignancy should be considered.
    (b) If tumor screening is recommended or desired:
        i. Abdominal ultrasound every 3 months until the third birthday then renal ultrasounds until the seventh birthday
        ii. Serum alpha-fetoprotein measurement every 3 months until the third birthday
        iii. Physical examination every 6 months by a medical professional until the seventh birthday

BWSp, Beckwith-Wiedemann spectrum; *ILO*, isolated lateralized overgrowth; *LO*, lateralized overgrowth.

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included in the decision-making process regarding tumor screening—especially in individuals who fall into the ILO category—because frequent screening studies and blood draws necessary for tumor surveillance can be burdensome. For individuals with LO who have an incomplete molecular evaluation (and therefore unclear tumor risk), the BWSp tumor screening guidelines should be considered, and the affected individual should be followed longitudinally (Table 2).[22]

Abdominal and renal ultrasounds

Complete abdominal ultrasounds to screen for hepatoblastoma and Wilms tumor are recommended every 3 months until the third birthday and then renal ultrasounds every 3 months until the seventh birthday for patients with ILO due to BWSp.[22] We also recommend at least 1 baseline abdominal ultrasound in individuals with idiopathic ILO because renal asymmetry can be useful in estimating tumor risk. In children with ILO who have undergone comprehensive molecular testing and who have symmetric kidneys (<1-cm difference in size), tumor risk can be assumed to be <1%, and ongoing screening may not be indicated. The decision on whether to pursue tumor screening in these cases needs to be based on the family's preference and level of comfort. However, renal asymmetry (≥1-cm difference) should raise concern that tumor risk may be >1%, and ongoing screening for malignancy would therefore be recommended as outlined above and in Table 2.

If there is broader ILO (any overgrowth extending beyond the wrist or ankle or including the trunk), it is recommended to follow the BWSp tumor screening guidelines[22] even in the absence of a known molecular cause because the available data do not provide evidence that the tumor risk in these individuals with ILO is <1%.

AFP measurements

These recommendations include serum AFP measurements every 3 months until the third birthday.[22] In many instances and particularly in the first years of life, AFP is elevated in patients with BWSp. Existing data sets on the natural history of age-specific AFP levels in BWSp patients vs the general population demonstrate persistently elevated levels that consistently decrease over time and normalize by age 13 months.[78] Persistent and significant increases in AFP levels over 2 measurements spaced 6 weeks apart should prompt an imaging evaluation for hepatoblastoma per guidelines.[12,22]

Caretaker abdominal examinations

We removed the recommendation of daily abdominal exams by the caretaker based on discussions with families about the burden and worry this places on them.

Conclusion

We have emphasized in this clinical practice resource that a thorough molecular evaluation should be performed before an individual is diagnosed with ILO. If this molecular evaluation is negative, the patient can be given the diagnosis of idiopathic ILO. The diagnostic process for conditions characterized by LO is complicated by phenotypic overlap and mosaicism. Although skin, tissue, or organ biopsies may seem invasive, the ramifications to clarify medical management are significant if an alternate overgrowth syndrome is identified that may obviate frequent tumor screening. Furthermore, emerging targeted medical therapies for several conditions in this category (that require molecular diagnosis) emphasize the utility of a molecular diagnosis, including the testing of affected tissues whenever possible. In addition, because the identification of a molecular diagnosis often reduces the need for tumor screening, it can also decrease the financial burden to the family and to health care systems. Finally, knowing a precise diagnosis enables more accurate estimation of tumor risk that can significantly decrease parental anxiety. We believe that identifying a molecular etiology and engaging parents in the diagnostic process are important so they can make informed decisions when being asked to allow invasive diagnostic procedures on their child. Individuals presenting with LO should be evaluated by a health care professional familiar with somatic overgrowth conditions because expertise can enhance the diagnostic workup and decision making with regard to tumor screening and available therapeutic options.

Ethics Declaration

Patient photos were acquired during the course of normal clinical practice, and all patients were consented under the CHOP IRB protocol 13-010658, in which they provided written consent to our team to use photographs in medical journal publications. In addition, each of these families completed a separate media release form in which they provided approval for the use of the image of the patient for medical journal publications on overgrowth conditions.

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Conflict of Interest

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Additional Information

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Supplemental material

Isolated lateralized overgrowth and the need for tumor screening: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG)

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Supplemental Table 1: Literature search terms

Literature search was conducted in Ovid MEDLINE (January 2008 – October 2021) using the following search terms: articles that contained any of the search terms A **and** search term B in title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, or synonyms. The number of articles identified when using the search terms separately is listed in parenthesis behind the search term. The number of articles that met criteria when searching for the combination of search term A **and** B was 940. Only articles written in English were included.

Search terms A (# of results)		**Search term B (# of results)**
hemihypertroph* (748)		
hemihyperplas* (143)		
((isolated or asymmetric or laterali#ed) adj3 overgrowth) (123)		
Beckwith-Wiedemann Syndrome (1164)	**AND**	(exp Neoplasms/ or (neoplasm* or cancer* or malignan* or neoplasia* or tumo?r?).mp.) adj5 (risk or prevalen* or predispos*) (325456)
(Beckwith-Wiedemann Syndrome or (beckwith wiedemann syndrome or beckwith-wiedemann syndrome or emg syndrome* or exomphalos macroglossia gigantism syndrome or exomphalos-macroglossia-gigantism syndrome* or wiedemann beckwith syndrome* or		

wiedemann syndrome* or wiedemann-beckwith syndrome* or "beckwith wiedemann" or "beckwith-wiedemann")) (1947)		
PIK3CA (6029)		
Proteus Syndrome (400)		
(Proteus Syndrome or (elephant man disease or proteus like syndrome or proteus syndrome or proteus-like syndrome)) (611)		
Hamartoma Syndrome, Multiple (1148)		
(bannayan riley ruvalcaba syndrome or bannayan zonana syndrome or bannayan-riley-ruvalcaba syndrome or bannayan-ruvalcaba-riley syndrome or bannayan-zonana syndrome or cerebellum dysplastic gangliocytoma or cerebellum dysplastic gangliocytomas or cowden disease or cowden syndrome or cowden's disease or cowden's syndrome or cowdens disease or cowdens syndrome or "dysplastic gangliocytoma of cerebellum" or "dysplastic gangliocytoma of the		

cerebellum" or lhermitte duclos disease or lhermitte-duclos disease or "macrocephaly, multiple lipomas, and hemangiomata" or "macrocephaly, pseudopapilledema, and multiple hemangiomas" or "macrocephaly, pseudopapilledema, and multiple hemangiomata" or multiple hamartoma syndrome or multiple hamartoma syndromes or myhre riley smith syndrome or myhre-riley-smith syndrome or pten hamartoma* or riley smith syndrome or riley-smith syndrome or ruvalcaba myhre smith syndrome or ruvalcaba-myhre syndrome or ruvalcaba-myhre-smith syndrome) (1609)		