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# Nephroblastomatosis or Wilms Tumor in a Fourth Patient with a Somatic PIK3CA Mutation

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Nephroblastomatosis or Wilms Tumor in a Fourth Patient with a Somatic PIK3CA Mutation

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Abstract

Wilms tumor and nephroblastomatosis are associated with syndromic conditions including hemihyperplasia. Hemihyperplasia is genetically heterogeneous and may be the result of genomic abnormalities seen in Beckwith-Wiedemann syndrome, mosaic chromosome or genomic abnormalities, or somatic point mutations. Somatic missense mutations affecting the PI3K-AKT-MTOR pathway result in segmental overgrowth and are present in numerous benign and malignant tumors. Here we report a fourth patient with asymmetric overgrowth due to a somatic PIK3CA mutation who had nephroblastomatosis or Wilms tumor. Similar to two of three reported patients with a somatic PIK3CA mutation and renal tumors, he shared a PIK3CA mutation affecting codon 1047, presented at birth with asymmetric overgrowth and had fibroadipose overgrowth. Codon 1047 is most commonly affected by somatic mutations in PIK3CA-related overgrowth spectrum (PROS). While the fibroadipose overgrowth phenotype appears to be common in individuals with PIK3CA mutations at codon 1047, individuals with a clinical diagnosis of Klippel-Trenaunay syndrome or isolated lymphatic malformation also had mutations affecting this amino acid. Screening for Wilms tumor in individuals with PROS-related hemihyperplasia may be considered and, until the natural history is fully elucidated in larger cohort studies, may follow guidelines for Beckwith-Wiedemann syndrome or isolated hemihyperplasia. It is not known if the specific PIK3CA mutation, the mosaic distribution or the clinical presentation affect the Wilms tumor or nephroblastomatosis risk in individuals with PROS.
Key Words: Wilms tumor, nephroblastomatosis, PIK3CA-related overgrowth, hemihyperplasia, hemihypertrophy, CLOVES, lipoma, somatic mutation
INTRODUCTION

Overgrowth syndromes can be associated with an increased risk for malignant tumors. Beckwith-Wiedemann syndrome is a typical example of this association [Shuman et al., 2000]. Asymmetric overgrowth or hemihyperplasia occurs in patients with Beckwith-Wiedemann syndrome (MIM 130650), but can be an isolated finding. An increased risk for malignant tumors, particularly for Wilms tumor, has been documented in individuals with Beckwith-Wiedemann syndrome or isolated hemihyperplasia (MIM 235000). Hemihyperplasia is genetically heterogeneous, including genomic abnormalities seen in Beckwith-Wiedemann syndrome, as well as mosaic chromosome or genomic abnormalities and somatic point mutations. Somatic mutations affecting the PI3K-AKT-MTOR pathway result in segmental overgrowth and other physical findings. Similarly, somatic mutations affecting the PI3K-AKT pathway are present in numerous benign and malignant tumors (see [Samuels and Waldman, 2010] for review). In individuals presenting in early childhood with segmental overgrowth or other findings related to somatic mutations in the PI3K-AKT-MTOR pathway, the mutation inherently occurred during an early developmental stage and may result in an increased lifetime risk for neoplasias driven by mutations in this pathway. Detailed understanding of the clinical phenotypes related to these mutations [Lee et al., 2012; Lindhurst et al., 2012; Poduri et al., 2012; Rios et al., 2012; Riviere et al., 2012; Kurek et al., 2012; Mirzaa et al., 2013; Keppler-Noreuil et al., 2014; Keppler-Noreuil et al., 2015] may allow for delineation of the associated cancer risks based on the specific mutation and the affected cell lineages. Here we report two individuals with somatic mosaicism for the most common PIK3CA mutations, c.3140A>G p.His1047Arg and c.3140A>T p.His1047Leu, and a history of Wilms tumor or
nephroblastomatosis and compare their presentations to the two other reported individuals with a somatic \textit{PIK3CA} mutation and Wilms tumor or nephroblastomatosis.

\textbf{MATERIALS AND METHODS}

Patient 1 was evaluated clinically and testing for overgrowth was completed clinically. Informed signed consent was obtained, and clinical data, clinical photographs and molecular results were reviewed. Patient 2 was enrolled in a somatic overgrowth study and evaluated at the National Institutes of Health after obtaining informed consent. She was previously reported as patient 23 in Keppler-Noreuil et al. [2014]. Updated history, exam and testing were obtained for this report.

We reviewed the literature and reviewed cohort data in order to gather information on the frequency of Wilms tumor and nephroblastomatosis in individuals with somatic \textit{PIK3CA} mutations.

\textbf{Cambridge cohort}

This study was approved by the UK National Research Ethics Committee. Written informed consent was obtained from all participants or their parents. Genomic DNA was extracted from lesions using standard procedures and imaging was conducted as part of routine clinical care. Somatic \textit{PIK3CA} mutations were detected in affected tissues using Next Generation Sequencing with preceding target enrichment. Equipment and materials were purchased from Life Technologies, Thermo-Fisher Scientific using a custom-designed primer pool which provides coverage of all coding regions of \textit{PIK3CA} and related genes (primer sequences available on
request). The mean depth of coverage for sequencing was 2000X. Mutations were verified to be
disease-causing on the basis of; i) finding the same mutation in additional probands with a
similar phenotype ii) published experimental data confirming activation of downstream
effectors of PI3K, and/or iii) the presence of the mutation in the catalogue of somatic mutations
in cancer (COSMIC) [Forbes et al., 2015].

Clinical Reports

Patient 1

The proband was born vaginally at 35 weeks of gestation after a pregnancy complicated by
maternal urinary tract infection and possible polyhydramnios on prenatal ultrasound. His G2P1-
>2 mother was 22 years old and his father was 26 years old. His African American parents were
non consanguineous. One maternal and three paternal half-sibs were in good health. Birth
weight was 2.47 kg (25-50\textsuperscript{th} centile for gestational age) and length 48.3 cm 75\textsuperscript{th} centile for
gestational age). OFC was not documented. Asymmetric overgrowth with right thigh
enlargement was present from birth and resulted in evaluations for hemihyperplasia. At age 5
months, physical examination was remarkable for bilateral supranumerary nipples, increased
girth in the right leg compared to the left, and a hypopigmented lesion on the lower abdomen.

A small left kidney with enlargement of the right kidney was noted on ultrasound at age
5 weeks. Renal ultrasound at age 9 months showed three well defined hypoechoic avascular
masses in the right kidney measuring 1.3x1.1x1.4 cm, 1.6x1.4x1.4 cm and 1.7x1.3x1.4 cm,
respectively (Figure 1a). The lesions were confirmed by CT study (Figure 1b, c), which
incidentally showed a marked asymmetry of the paravertebral and pelvic musculature with all
muscles on the right larger than the on the left. The MRI imaging similarly demonstrated the renal masses (Figure 1d). Wilms tumor was diagnosed and chemotherapy administered in an effort to allow surgery later with preservation of kidney function. This decision was in consideration of his atrophic left kidney, which contributed 23% to total renal function. A subsequent CT scan (Fig. 1c) demonstrated the tumors’ sizes to be unchanged or increased, and a needle biopsy was performed at age 10 months. In this post-treatment biopsy the pathology diagnosis was a nephrogenic lesion, it was impossible to differentiate between nephrogenic rests and Wilms tumor. Chemotherapy was completed as planned. The lesions responded to therapy and were monitored through imaging studies. Surgical resection was not performed.

A soft lipomatous mass in the right paraspinal region above the iliac crest was first documented at age 2 years. An MRI at age 7 years showed a 2.8x2.8x10 cm focus of abnormal signal intensity within the right vastus lateralis muscle. An MRI at age 7 10/12 years showed the same stable lesion and regions of post contrast enhancement in the right iliac muscle (Figure 2, a-d). Fatty lobules in the left paraspinal soft tissue at L3-L5 appeared stable compared to previous studies and did not encroach on the neural foramina. The right L3 root was anteriorly displaced, implying fatty infiltration in the right L3-L4 neural foramen. Stable fatty prominence in the right L5-S1 neural foramen was noted. Due to discomfort, the paraspinal intramuscular lipoma was surgically removed at age 8 years. Pathology showed mature adipose tissue encompassing skeletal muscle, consistent with an intramuscular lipoma.

The size discrepancy of his legs persisted. An enhancing lesion in the right psoas was seen on MRI (Figure 2b) and a needle biopsy results obtained at age 4 years suggested an inflammatory myopathy. The entire right leg was larger than the left in diameter and the right
femur was approximately 1 cm longer than the left at age 7 years. The patient had a mild gait
abnormality owing to the asymmetric overgrowth. Persistent thigh pain was the indication for
an MRI showing signal abnormalities in the vastus lateralis and peroneus brevis (Figure 2 c, d),
but open muscle biopsy at age 8 years showed skeletal muscle without significant pathology.

His motor and speech development were age appropriate with walking independently
at age 1 year and single words around the same time. At age 7 years his cognitive development
was age appropriate and he attended a typical classroom setting. His height at age 7 years was
127 cm (50<sup>th</sup>-75<sup>th</sup> centile), weight 33.7 kg (>97<sup>th</sup> centile, Z-score 1.8) and OFC 52.5 cm (50<sup>th</sup>-75<sup>th</sup>
centile). His facial features were symmetric and non dysmorphic. A large mass was visible in the
right paraspinal region (Figure 3a), and the leg size discrepancy was striking (Figure 3b, c).

Molecular Laboratory Study Results

An overgrowth panel was performed clinically on a next generation platform at the
University of Pennsylvania. Testing included site-specific regions for AKT1, AKT2, AKT3, GNAQ,
MTOR, PIK3CA and PIKR2; in addition the coding and flanking intronic boundaries for CDKN1C
were covered.

DNA samples derived from a frozen psoas muscle tissue contained a mosaic
heterozygous (12.9-15.7%) PIK3CA mutation, c.3140A>G, in exon 20, predicting a p.His1047Arg
amino acid substitution. The same mutation was present as mosaic heterozygote (21.4-24.8%)
in a DNA sample from a tissue block of the right thigh mass. Blood sample derived DNA did not
show the mutation with a confidence score of 99.99%, leading to the conclusion that the
mutation occurred somatically.
Additional tests performed with non-diagnostic results included methylation-sensitive multiplex ligation-dependent probe amplification for large deletions, duplications and/or methylation abnormalities in the IC1 (*H19*) and IC2 (*LIT1*) critical regions on 11p15 associated with Beckwith-Wiedemann syndrome.

**Patient 2**

This individual was originally reported as patient 23 in Keppler-Noreuil et al. [2014] and clinical information was updated at age 10 years. The proposita was born at 38 weeks’ gestation by induced vaginal delivery after a pregnancy complicated by abnormal prenatal ultrasounds revealing “webbed toes” on her right foot and “white spots” on her heart. Her G1P0-1 mother was 21 years and her father was 26 years old. Her African American parents were nonconsanguinous and subsequently had a healthy son. Birth weight was 2.95 kg (10-25\textsuperscript{th} centile) and length 49.5 kg (50\textsuperscript{th} centile). Her right leg and foot, including her toes were noted to be enlarged, and she had hyperpigmentation of her neck and waist.

At age 2 years, she was found on renal ultrasound imaging to have bilateral hypodense renal lesions. She was diagnosed with Wilms tumor. She was treated with 4 months of chemotherapy with vincristine, actinomycin, and adriamycin followed by bilateral partial nephrectomies. Pathology from the nephrectomies showed adenomatous nephrogenic rests. Her renal function has been normal. At age 10 years, abdominal ultrasound showed stable size asymmetry of the kidneys with no change in the moderate hydronephrosis involving the right kidney; parenchymal thinning with increased cortical echogenicity of the right kidney, and normal corticomedullary differentiation of the left kidney with an unchanged nephrogenic rest.
She has had progressive overgrowth of her right leg and foot with leg length
discrepancy. She has undergone multiple orthopedic surgeries including right foot Boyd
amputation at age 2 years and epiphysiodesis of the right femur and hemiepiphysiodesis of the
right tibia at age 4 years. An MRI scan of her lower extremities at 6 years showed diffuse multi-
compartmental lipomatosis of the lower extremities with muscular infiltration, right greater
than left with right buttock and leg enlargement and muscle atrophy. Right patellar dislocation
was present associated with the intra-articular lipomatosis. Liposuction of her right leg was
performed at age 6 years, and debulking of her right knee at 7 years. She had eight-plate
removal from the right lateral proximal tibia, medial distal femur and lateral distal femur at 7
years. She underwent laparoscopic surgery to remove excess subcutaneous fat from the right
side of her abdomen at age 8 years.

Her motor and speech development were apparently normal. She crawled at 6 months,
walked at 10 months, drank from a cup at 11 month, spoke in 2 word sentences at 12 months,
toilet trained at 14 months, and was riding a 2 wheel bike at 8 years. She was in a regular class
setting with additional help in math. She was diagnosed with attention deficit disorder at age 9
years.

On examination at 10 years, her height was 139.2 cm (50-75\textsuperscript{th} centile), weight 45.4 kg
(90-97\textsuperscript{th} centile) and OFC 54.2 cm (85\textsuperscript{th} centile). Tanner III breast development was noted. She
had marked increased, asymmetric enlargement of the right leg and right buttock since her
initial examination, and a right flank pigmented nevus extending from her waist to her pelvis
bilaterally. She had soft tissue overgrowth of her left lower and right abdomen and
enlargement of her left labia majora. She ambulated with a right Syme prosthesis. Fat tissue appeared reduced at the arms, upper torso, and face with prominent muscles and vasculature. Thigh circumference was right 61 cm, left 38.8 cm; calf circumference: right 37.5 cm, left 25.2 cm. Feet length: right amputated, left 24.6 cm. Right hip was lower than the left. Her left great toe was enlarged and laterally deviated (Figure 4,a-d).

Molecular Laboratory Study Results

Molecular analysis consisted of candidate mutation analysis for somatic mutations in \textit{PIK3CA} using a custom PCR restriction assay as described in Lindhurst et al. [2012] for the c.3140A>T p.His1047Leu mutation. This mutation was found in adipose and skin samples from the left leg at the level of 3-4%, with negative results from peripheral blood. 

\textit{PTEN} mutation analysis was normal.

RESULTS

Literature Review and Cohort Data

Literature review revealed two reports including individuals with a somatic \textit{PIK3CA} mutation and Wilms tumor [Kurek et al., 2012; Luks et al., 2015] (Table I). Keppler-Noreuil et al. [2014] reviewed the clinical and natural history of \textit{PIK3CA} related overgrowth spectrum in a cohort of 35 individuals (Table II), including one reported here with updated history and findings as Patient 2. Further, two patients with Wilms tumor and megalencephaly-capillary malformation (MCAP) (602501) syndrome were reported prior to the identification of the molecular basis of MCAP [Lapunzina et al., 2004; Wright et al., 2009].
In a combined cohort of 159 individuals with somatic mutations in *PIK3CA* from the Seattle Children’s Research Institute and the University of Cambridge, UK, no individual was identified with Wilms tumor or nephroblastomatosis (Table II). However, these individuals had a broad spectrum of clinical phenotypes, including CLOVES syndrome (612918), MCAP, fibroadipose overgrowth and isolated hemihyperplasia or macrodactyly. It is important to note that longitudinal follow-up data are not available on all individuals and formalized tumor screening by abdominal imaging has not been performed on all. The individuals from the UK ranged in age from one to 57 years (mean of 16 years), and 45 individuals have had formalized screening with abdominal imaging (ultrasound, MRI, CT scan). Overlap of the Cambridge cohort with that reported by Keppler-Noreuil et al. [2014] is noted in Table II.

**DISCUSSION**

The two patients described here were diagnosed with renal masses at 9 months and 2 years of age, respectively. Imaging studies in both identified hypodense masses in the kidneys suggestive of Wilms tumor. In the first patient, pathology from needle biopsy performed after treatment was indeterminate regarding the diagnosis of Wilms tumor versus nephrogenic rests. In the second patient, pathology showed adenomatous nephrogenic rests. Both patients were treated with chemotherapy, and their follow up studies have been stable. Very few patients with hemihyperplasia due to a somatic *PIK3CA* mutation and Wilms tumor or nephroblastomatosis have been reported (Table I). Nephrogenic rests or nephroblastomatosis refer to foci of embryonal cells persisting beyond 36 weeks of gestation and capable of developing into nephroblastomas (Wilms tumor) [Murphy et al., 2004]. These are found in
approximately 1% of infant kidneys at autopsy and are associated with an increased risk of Wilms tumor [Lonergan et al., 1998]. Nephroblastomatosis is associated with syndromes including Beckwith-Wiedemann syndrome, isolated hemihyperplasia, chromosomal abnormalities and aniridia [Scott et al., 2006a]. These precursors of Wilms tumor are encountered in 25-40% of patients with Wilms tumors. They are often considered a spectrum lesion and, like in Patient 1 reported here, cannot always be distinguished. Perilobar nephroblastomatosis is typically treated with chemotherapeutic, as was done in Patient 1.

Kurek et al. [2012] described a female with a clinical diagnosis of CLOVES syndrome and a history of Wilms tumor. She had lipomatous overgrowth of the trunk and limbs, with wide feet and polydactyly in addition to striking overgrowth of both legs. This patient was mosaic for the PIK3CA p.His1047Arg mutation in her legs, but negative for the mutation in a saliva-derived DNA sample. Another individual with CLOVES and Wilms tumor was mosaic for the PIK3CA p.Asn345Lys mutation and limited clinical information was available (Table I) [Luks et al., 2015].

Neither the individuals reported here nor the patient in Kurek et al. [2012] had megalencephaly. This is noteworthy because the phenotypic spectrum associated with \textit{PIK3CA} mutations encompasses MCAP syndrome, a clinically distinct disorder manifesting predominantly with severe brain overgrowth, and milder body overgrowth than other \textit{PIK3CA}-related disorders [Mirzaa et al., 2013]. In a series of 12 patients with MCAP, one had a Wilms tumor [Wright et al., 2009]. This 4-year-old male patient had lipomas and a dermatomyofibroma. He did not show a \textit{PTEN} mutation, no other testing was reported [Wright et al., 2009]. Another patient with MCAP and Wilms tumor has been reported [patient 2, Lapunzina et al., 2004]. This 10 month old girl had megalencephaly, hydrocephalus, cutaneous
vascular malformations, joint hyperlaxity, asymmetry and 2-3 toe syndactyly [Lapunzina et al., 2004]. We are not aware whether this patient has been tested for PIK3CA mutations.

Somatic PIK3CA Mutation Associated Phenotypes

Phenotypes associated with somatic PIK3CA mutations are extremely variable, depending upon the timing and location of their postzygotic occurrence and the effect of the specific amino acid on the protein product. This phenotypic spectrum is now referred to as PIK3CA-Related Overgrowth Spectrum (PROS) [Mirzaa et al., 2013; Keppler-Noreuil et al., 2014] and encompasses a number of originally clinically defined conditions. The MCAP syndrome was previously known as macrocephaly- cutis marmorata teleangiectasia congenita or macrocephaly-cutis marmorata and is primarily distinguished by brain overgrowth (megalencephaly or hemimegalencephaly) with associated neurologic complications (hydrocephalus, Chiari malformation), cutaneous capillary malformations with focal or generalized somatic overgrowth and syndactyly or polydactyly, as well as variable connective tissue dysplasia [for review see Mirzaa et al., 2013]. The CLOVE syndrome is defined by congenital lipomatous overgrowth, vascular malformations and epidermal nevi [Sapp et al., 2007] and shows significant overlap with fibroadipose hyperplasia. The acronym was extended to CLOVES in order to account for skeletal anomalies, scoliosis, spinal anomalies and seizures [Alomari 2009]. The CLOVES syndrome may be differentiated from MCAP by the severity of somatic overgrowth with characteristic overgrowth of lipomatous tissue and high risk of lymphatic and vascular malformations in the former; whereas brain overgrowth predominates in MCAP and somatic manifestations, while present, are typically milder than in CLOVES
syndrome. Skeletal anomalies including scoliosis and macrodactyly may be more prominent in CLOVES syndrome, but polydactyly and syndactyly occur in both syndromes. Individuals having overlapping findings of both syndromes are increasingly recognized. Overlap of phenotypic findings between CLOVES syndrome and MCAP is exemplified by the extensive cutaneous involvement of a truncal vascular malformation in Patient 3 reported by Sapp et al., [2007] and the individual reported by Gucev et al. [2008], who had features of CLOVES syndrome and hemimegalencephaly. Vascular malformations affecting the skin in combination with focal overgrowth are characteristic for Klippel-Trenaunay syndrome, and PIK3CA mutations were found in 3/15 patients clinically diagnosed with Klippel-Trenaunay syndrome [Kurek et al., 2012]. In the majority of patients with Klippel-Trenaunay syndrome, isolated lymphatic malformation or a combination of findings including fibro-adipose vascular anomalies, mutations in PIK3CA were identified [Luks et al., 2015]. A wide range of unusual presentations has been described in case reports, including unilateral hand muscle overgrowth [Castiglioni et al., 2014], segmental overgrowth syndrome [Rasmussen et al., 2014] and mesenteric lipomatosis [Cohen et al., 2014]. The most common mutation is a postzygotic change affecting amino acid 1047, with p.His1047Arg in 19 and p.His1047Leu in 8 of 35 individuals reviewed by Keppler-Noreuil et al. [2014] (Table II). While the CLOVES syndrome or fibroadipose hyperplasia phenotype appears to be common in individuals with a missense mutation at codon 1047, individuals with a clinical diagnosis of Klippel-Trenaunay syndrome or isolated lymphatic malformation [Kurek et al., 2012; Luks et al., 2015] also had a mutation affecting this amino acid. Keppler-Noreuil et al. [2014] differentiated between mutations at p.His1047, which affect the catalytic domain of the protein product, and multiple other changes in the coiled domain.
and concluded that the majority of patients with CLOVES syndrome had mutations in the latter. While this was statistically significant within their cohort, there were exceptions even within their relatively small cohort.

Somatic PIK3CA Mutation Associated Malignancies and Wilms Tumor

The catalytic subunit of phosphatidylinositol-3-kinase (PI3K) is somatically mutated in many cancers including colorectal, ovarian, breast, hepatocellular carcinomas and glioblastomas. These PIK3CA mutations are located mostly at hotspots within the kinase domain (encoded by exon 20), and result in gain-of-function implicated in oncogenicity [Samuels et al., 2004; Ikenoue et al., 2005; Kang et al., 2005]. However, isolated Wilms tumor has not previously been reported in association with somatic PIK3CA mutations. The risk of tumorigenesis, including Wilms tumor, in patients with isolated hemihyperplasia ranges from 3.3-6% [Hoyme et al., 1998; Lapunzina 2005; Clericuzio and Martin, 2009]. Wilms tumor has been reported in four individuals with phenotypes associated with somatic PIK3CA mutations, including the two described here.

Wilms Tumor Screening Recommendations

Screening has been recommended for young children with syndromic conditions encompassing an increased risk for Wilms tumor, most classically Beckwith-Wiedemann syndrome [Beckwith 1998; Choyke et al., 1999; Clericuzio and Martin, 2009]. Based on the perceived difference in the tumor risk ranging from high in isolated hemihyperplasia to mild or moderate in Klippel-Trenaunay and macrocephaly- capillary malformation syndrome[Table XVIII in Lapunzina 2005], varying recommendations for tumor screening have been proposed [Lapunzina 2005].
Lapunzina [Table V in Lapunzina 2005] reviewed the screening guidelines for multiple
overgrowth syndromes and as expected, the abdominal ultrasound recommendations were
identical for isolated hemihyperplasia and Beckwith-Wiedemann syndrome with screening
every 3 months until age 4 years, every 6 months until age 7 years and annually thereafter. In
contrast, it was recommended that individuals with MCAP receive an annual abdominal
ultrasound in all age groups [Lapunzina, 2005]. The American College of Medical Genetics
practice guidelines for Wilms tumor screening in patients with isolated hemihyperplasia suggest
abdominal ultrasound every 3 months until age 7 years [Clericuzio and Martin, 2009]. No
abdominal ultrasound was recommended for individuals with Klippel-Trenaunay syndrome,
based on Green et al.’s [2004] review of 115 patients with Klippel-Trenaunay who did not
develop Wilms tumor and a study cohort of 8614 individuals with Wilms tumor, none of which
had Klippel-Trenaunay. While there was one report each of bilateral Wilms tumor [Ehrich et al.,
1979] and bilateral nephroblastomatosis [Mankad et al., 1974] in individuals with Klippel-
Trenaunay syndrome, no recent reports of this association have been published. Importantly,
these screening recommendations [Green et al., 2004; Lapunzina 2005] were published before
the molecular characterization of Klippel-Trenaunay syndrome, and the clinical diagnosis of
Klippel-Trenaunay syndrome is not always consistently defined, making it difficult to determine
whether reported individuals actually had PROS or another overlapping disorder. Some patients
with hemihyperplasia have an underlying somatic \textit{PIK3CA} mutation and their increased risk for
Wilms tumor, and possibly other embryonal tumors, may be at least partially accounted for by
the \textit{PIK3CA} mutation. Given the prevalence of \textit{PIK3CA} mutations affecting codon 1047 in
cancer, a critical consideration is whether patients with these particular mutations are at
increased risk of Wilms tumors. Including the cases reported here, four individuals with
documented \( \text{PIK3CA} \) mutations and Wilms tumor or nephroblastomatosis were reported. The
combined number of patients with documented \( \text{PIK3CA} \) mutations in the literature as
calculated in Table II and after adding Patient 1 reported here is 258. Considering
ascertainment bias for the Patient 1 in this report it is likely that the risk for Wilms tumor or
nephroblastomatosis in individuals with \( \text{PIK3CA} \) mutations is less than the calculated 4/258 or
1.6%. While this risk is increased compared to the general population, it does not meet the 2-
5% risk suggested by Scott et al. [2006b] in order to warrant screening studies. However, in light
of the clearly increased risk and the variable preferences of families and medical care providers
in different care environments, we consider screening by ultrasound appropriate, similar to the
recommendations for hemihyperplasia [Clericuzio and Martin, 2009], as indicated in Table III.
Because many individuals with PROS have overgrowth, the screening guidelines for
hemihyperplasia [Clericuzio and Martin, 2009] would be applied prior to the identification of a
\( \text{PIK3CA} \) mutation.

Three patients with PROS and Wilms tumor or nephroblastomatosis had somatic
mutations affecting \( \text{PIK3CA} \) codon 1047, which is associated with oncogenicity in isolated
cancers. Both patients described here in more detail had extensive overgrowth involving the
legs and trunk. Although this evidence is not sufficient to demonstrate high risk, it would be
prudent to consider serial abdominal ultrasounds in patients with a somatic \( \text{PIK3CA} \) mutation
similar to the recommendations for isolated hemihyperplasia and Beckwith–Wiedemann
syndrome. More longitudinal data including clinical examination and regular screening studies
are needed on patients with PROS due to different \( \text{PIK3CA} \) mutations, in order to accurately
determine risk of tumorigenesis. Screening recommendations may then possibly be stratified based on the specific mutation or the clinical presentation.

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PIK3CA RefSeq NM_006218.2
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145:287-293.
Legends:

Figure 1: Ultrasound of Patient 1 obtained at age 9 months, showing multiple hypoechoic, avascular right renal masses in the lower pole and interpolar region (a); contrast enhanced CT scans at age 9 months (b) and 12 months (c) show well-defined low attenuation lesions enlarging in size in the hypertrophied right kidney. The left kidney is atrophied. (c) MRI demonstrates well-defined lesions with mild heterogeneous internal enhancement.

Figure 2: Patient 1’s MRI obtained at age 7 years showed (A) a prominent right paraspinal fatty mass involving erector spinae musculature with anterior displacement of the right L3 exiting nerve root implying fat infiltration into the right L3-L4 neural foramen; increased diameter of the right psoas, thigh and calf musculature compared to the left (not shown) with enhancing lesions in the right psoas (B), vastus lateralis and medial right thigh musculature (C), and in the peroneus brevis (D).

Figure 3: (A) Patient 1’s back at age 7 years, showing protrusion from lipoma over right lower back, (B, C) Patient 1’s legs note overgrowth of right leg, most obvious in the right thigh.

Figure 4: Patient 2 at age 10 years, frontal view (A), showing reduced subcutaneous fat in her face, arms and chest, and lipomatous overgrowth of her left>right abdomen, right leg and left ankle, (B) back view, (C) closer view of her legs with overgrowth of right leg s/p right Syme amputation and lipomatous mass of her medial left ankle, (D) Closer view of her abdomen with masses involving the right upper abdomen, and left mid- to lower abdomen.
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Legend: CLOVES; Congenital lipomatous overgrowth with vascular, epidermal and skeletal anomalies
Table II: Wilms Tumor or Nephroblastomatosis in Cohorts of Patients with Somatic PIK3CA Mutations

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<th>PIK3CA amino acid change</th>
<th>Seattle cohort</th>
<th>Cambridge cohort</th>
<th>Keppler-Noreuil et al. [2014]</th>
<th>Luks et al., [2015] Table II</th>
<th>Combined number of individuals</th>
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*Includes # patients from Cambridge cohort: 1 E545K, 5 H1047L, 4 H1047R. These patients have had follow up screening in the Cambridge cohort.

** This is less than the cohorts combined because 10 patients were included in Keppler-Noreuil et al. [2014] and in the Cambridge cohort.
Table III: Imaging Recommendations for Patients with PIK3CA-Related Overgrowth Spectrum (PROS)

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Concern or indication</th>
<th>Suggested imaging study</th>
<th>Timing of initial imaging study</th>
<th>Timing of subsequent imaging studies</th>
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<tr>
<td>Brain, Facial</td>
<td>Ventriculomegaly, hydrocephalus, Chiari malformation/cerebellar tonsillar ectopia, cortical brain malformations (polymicrogyria)</td>
<td>Brain MRI without contrast</td>
<td>At diagnosis, if there is macrocephaly (OFC &gt; 2 SD), developmental delay, epilepsy, facial or skull involvement</td>
<td>As indicated based on results of previous studies or when symptomatic</td>
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<td>Spinal canal</td>
<td>Tethered cord, Syringomyelia, Lipomeningocele</td>
<td>Ultrasound in infant; MRI thereafter</td>
<td>At diagnosis if truncal involvement present</td>
<td>As indicated based on results of previous studies or when symptomatic</td>
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<td>Spine</td>
<td>Scoliosis</td>
<td>Spine radiographs</td>
<td>At presentation if spinal asymmetry or truncal overgrowth is noted</td>
<td>As indicated based on results of previous studies or when new onset scoliosis is suspected</td>
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<td>Trunk</td>
<td>For truncal overgrowth, scoliosis, lymphatic or vascular malformations</td>
<td>Whole body MRI, consider contrast as needed</td>
<td>Infants at 12 months (due to need for sedation); for older individuals at diagnosis</td>
<td>As indicated based on results of previous studies or when symptomatic</td>
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<td>Extremities</td>
<td>Overgrowth, asymmetry, lymphatic or vascular malformations, thromboembolism</td>
<td>Radiographs, MRI, Consider Doppler ultrasounds of involved arms, legs or both</td>
<td>At diagnosis of overgrowth affecting extremities</td>
<td>As indicated based on results of previous study; to monitor progression of overgrowth or to plan surgery</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Enlargement, tumor (Nephroblastomatosis or Wilms tumor),</td>
<td>Renal ultrasound</td>
<td>At diagnosis</td>
<td>Repeat every 3-4 months until age 8 years</td>
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Figure 1: Ultrasound of Patient 1 obtained at age 9 months, showing multiple hypoechoic, avascular right renal masses in the lower pole and interpolar region (a); contrast enhanced CT scans at age 9 months (b) and 12 months (c) show well-defined low attenuation lesions enlarging in size in the hypertrophied right kidney. The left kidney is atrophied. (c) MRI demonstrates well defined lesions with mild heterogeneous internal enhancement.

254x190mm (96 x 96 DPI)
Figure 2: Patient 1’s MRI obtained at age 7 years showed (A) a prominent right paraspinal fatty mass involving erector spinae musculature with anterior displacement of the right L3 exiting nerve root implying fat infiltration into the right L3-L4 neural foramen; increased diameter of the right psoas, thigh and calf musculature compared to the left (not shown) with enhancing lesions in the right psoas (B), vastus lateralis and medial right thigh musculature (C), and in the peroneus brevis (D).
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254x190mm (96 x 96 DPI)
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254x190mm (96 x 96 DPI)