Published in final edited form as:

Clin Genet. 2018 May ; 93(5): 1075–1080. doi:10.1111/cge.13195.

Causal somatic mutations in urine DNA from persons with the CLOVES subgroup of the PIK3CA Related Overgrowth Spectrum (PROS)

Megan E. Michel^{1,*}, Dennis J. Konczyk^{2,*}, Kit San Yeung³, Rudy Murillo¹, Matthew P. Vivero², Amber M. Hall⁴, David Zurakowski⁴, Denise Adams⁵, Anita Gupta⁶, August Y. Huang¹, Brian H.Y. Chung³, and Matthew L. Warman¹

¹Orthopaedic Research Laboratories, Department of Orthopaedic Surgery, Boston Children's Hospital, MA 02115

²Department of Plastic and Oral Surgery, Boston Children's Hospital, Boston, MA 02115

³Department of Paediatrics and Adolescent Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

⁴Department of Anesthesia, Boston Children's Hospital, Boston, MA 02115

⁵Division of Hematology/Oncology, Department of Medicine, Boston Children's Hospital, Boston, MA 02115

⁶Department of Pathology and Lab Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

Abstract

Congenital Lipomatous Overgrowth with Vascular, Epidermal, and Skeletal anomalies (CLOVES) and Klippel-Trenaunay (KTS) syndromes are caused by somatic gain-of-function mutations in *PIK3CA*, encoding a catalytic subunit of phosphoinositide 3-kinase. Affected tissue is needed to find mutations, since mutant alleles are not detectable in blood. Because some patients with CLOVES develop Wilms tumor, we tested urine as a source of DNA for mutation detection. We extracted DNA from the urine of 17 and 24 individuals with CLOVES and KTS, respectively, and screened 5 common *PIK3CA* mutation hotspots using droplet digital PCR. Six of 17 CLOVES participants (35%) had mutant *PIK3CA* alleles in urine. Among 8 individuals in whom a mutation had been previously identified in affected tissue, 4 had the same mutant allele in the urine. One study participant with CLOVES had been treated for Wilms tumor. We detected the same *PIK3CA* mutation in her affected tissue, urine, and tumor, indicating Wilms tumors likely arise from *PIK3CA* mutant cells in patients with CLOVES. No urine sample from a participant with KTS had detectable *PIK3CA* mutations. We suggest that urine, which has the advantage of being collected non-invasively, is useful when searching for mutations in individuals with CLOVES syndrome.

Correspondence to: Matthew L. Warman, MD, Orthopaedic Research Laboratories, Boston Children's Hospital, 320 Longwood Avenue, Boston, MA 02115, Phone 617-919-2371, matthew.warman@childrens.harvard.edu.

*these authors contributed equally

The authors declare they have no conflicts of interest.

Graphical abstract

Introduction

The *PIK3CA*-related overgrowth spectrum (PROS) represents a family of disorders caused by somatic mutations in *PIK3CA* (1). *PIK3CA* encodes a catalytic subunit of the class 1 phosphoinositide 3-kinase (PI3K), and PROS-causing mutations activate PI3K enzymatic activity (2). Among congenital syndromes currently falling under the PROS umbrella are CLOVES (Congenital Lipomatous Overgrowth with Vascular Epidermal and Skeletal anomalies) (3), FAO (Fibroadipose Overgrowth) (4), MCAP (Megalencephaly-Capillary malformation) (5), and Klippel-Trenaunay (KTS) (6). Localized disorders can also fall under the PROS umbrella, including lymphatic malformation (6, 7), macrodactyly (8), and hemimegalencephaly (9).

PROS-causing *PIK3CA* mutations are found in DNA recovered from affected tissue using technologies that detect mutant allele frequencies as low as 1-in-200 cells. Applying these methods, somatic disease-causing *PIK3CA* mutations have been identified in 67 to 95% of participants clinically suspected of having PROS (6, 10). When paired affected tissue-white blood cell (WBC) DNA samples have been studied, *PIK3CA* mutations are most often found only in the affected tissue (10). This may be due to strongly activating *PIK3CA* mutations being completely selected against by WBCs or their stem cell precursors. Therefore, WBC DNA cannot be used to search for mutations in most persons suspected of having PROS (1, 10). Not yet known is the rate of detecting PROS-causing mutations in other “unaffected” tissues, since few affected-unaffected tissue sample pairs have been analyzed. Thus, current methods for reliably detecting *PIK3CA* mutations in persons suspected of PROS require a biopsy or surgical collection of affected tissue. Screening DNA present in urine could enable non-invasive *PIK3CA* mutation identification, but previous studies of PROS patients have not searched for mutations in urine DNA (1, 10, 11).

Many somatic *PIK3CA* mutations that cause PROS are also common somatic mutations in cancer. For example, a somatic missense mutation affecting the 1047th amino acid residue of *PIK3CA* (p.H1047R) was found in 15/162 individuals with PROS (10) and in 160/1462 breast cancer specimens (cancer.sanger.ac.uk; accessed 7/27/2017) (12). Despite the frequent occurrence of somatic *PIK3CA* mutations in tumors, there are only a few case reports of individuals with mutation-confirmed PROS developing cancer (3, 6, 13, 14). This may be because of the short period of follow-up available for most patients with PROS or because *PIK3CA* missense mutations enhance tumor growth but do not cause cell transformation.

Wilms tumor has been confirmed in 4 children and nephroblastomatosis confirmed in 2 children with PROS who have somatic *PIK3CA* mutations (3, 6, 13, 14). In patients for whom clinical information has been published, most of these individuals' phenotypic features fit best with the CLOVES syndrome subgroup (3, 6, 13, 15, 16). Additional case reports of Wilms tumor arising in individuals with KTS (17, 18) and MCAP (19, 20) were published before CLOVES syndrome was described (21, 22) and before somatic *PIK3CA* mutations were associated with CLOVES (3), KTS (6), and MCAP (5). These observations led us to test whether urine DNA contains detectable *PIK3CA* mutations in individuals with the CLOVES and KTS subgroups of PROS. One of our study participants with CLOVES had previously had a Wilms tumor resected (3), thereby enabling us to determine whether this tumor is comprised of *PIK3CA* mutant cells.

Materials and Methods

Participant ascertainment

This study was approved by the Committee on Clinical Investigation at Boston Children's Hospital and by the Institutional Review Board at the University of Hong Kong. All study participants or their legal guardians provided informed consent/assent. Participants were invited to join the study during a CLOVES Family Meeting at Boston Children's Hospital, a K-T Support Group Meeting in Rochester MN, or during clinic visits to Boston Children's Hospital or Queen Mary Hospital. All 17 individuals with CLOVES syndrome were evaluated clinically at BCH or Queen Mary Hospital and diagnosed using published criteria (6, 23). Four out of 24 individuals with KTS were clinically evaluated at BCH or Queen Mary Hospital and satisfied previously published criteria (6). Medical records and imaging studies were unavailable for the remaining 20 individuals who provided urine samples at the K-T Support Group meeting; in these individuals the diagnosis of KTS was made based on clinical history. For the study participant who previously had a Wilms tumor that was surgically resected, flash frozen in liquid nitrogen, and stored at -80°C , a portion of the specimen was obtained from the treating hospital.

Sample collection, DNA extraction, and sequencing

Urine samples were collected in sterile containers and stored at 4°C . Specimens were transferred to 50 mL conical tubes and centrifuged for 15 min. at $6400 \times g$ (4°C) to pellet the cells. The pellet was resuspended in 200 μL PBS and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's directions. Samples with DNA concentrations below 5 ng/ μL were concentrated using either a speed vac or the DNA Clean & Concentrator-25 kit (Zymo Research). A portion of the frozen Wilms tumor specimen was bisected, with half being used for cryosectioning to confirm it predominantly contains tumor cells, and half being used for DNA extraction.

Droplet Digital PCR (ddPCR)

We previously reported ddPCR assays for 5 "hotspot" mutations (p.C420R, p.E542K, p.E545K, p.H1047R, and p.H1047L). These assays detected causal *PIK3CA* mutations in affected tissue from 30/33 individuals with CLOVES syndrome and 19/21 individuals with KTS (6). Therefore, we used these same ddPCR assays to screen DNA extracted from urine

and from the Wilms tumor. If 3 mutant allele-containing amplicons were detected in a urine DNA ddPCR assay or if a participant had a mutation previously identified using affected tissue DNA, then the urine DNA ddPCR assay was repeated. We also used WBC DNA from healthy individuals without PROS to determine the rate at which mutant amplicon-containing droplets appeared among the total droplets (~14,000) in an assay when only wild-type DNA was used as template (6). Furthermore, we used urine DNA samples from 8 persons with known *PIK3CA* mutations to determine the rate at which mutant amplicon-containing droplets appeared in ddPCR assays designed for the other mutations. After determining these “false positive” rates, we used the Poisson estimation to calculate the *p* value for observing a specified number of mutant amplicon-containing droplets in a ddPCR assay when no actual mutant alleles are present.

Determination of Renal-Urinary Involvement

Clinical records, including radiology reports of abdominal MRI and/or ultrasound studies, were reviewed for each participant with CLOVES syndrome. Presence of any of the following features/findings was considered evidence of Renal-Urinary system involvement: hydronephrosis, renal asymmetry or bilateral enlargement seen on imaging, bladder wall involvement observed by cystoscopy, or recurrent hematuria.

Results

We observed that when DNA from healthy individuals or DNA from individuals with a different *PIK3CA* mutation was used as template, each of the ddPCR assays yielded mutant amplicon-containing droplets at frequencies 0.008%. Using 0.008% as the rate of observing mutant amplicon-containing droplets when no mutant alleles are present, the probability of observing 3 and 6 mutant amplicon-containing droplets out of 14,000 total droplets is 0.10 and 0.001, respectively.

We identified *PIK3CA* hotspot mutations ($p < 0.05$) in urine DNA samples from 8 of 17 participants with CLOVES and 0 of 24 participants with KTS (Table).

In 6 of the 17 CLOVES participants, the observed numbers of mutant droplets remained significant after correcting for multiple hypothesis testing ($p < 0.0001$). For 8 participants with CLOVES, we had previously identified their causal *PIK3CA* mutation using ddPCR with DNA from affected tissue as template (6, 23). In 4 of these 8 individuals we detected the same mutation in their urine. We collected urine twice, a year apart, in one participant with CLOVES (individual CL-U11) and detected the same mutation each time. In another participant (individual CL-U09, Figure 1A), Wilms tumor was diagnosed and resected 26 months before the urine sample was collected. This individual had the same mutation in her affected tissue, urine, and Wilms tumor. Histologic assessment of her tumor specimen indicated it contained mostly tumor cells (Figure 1B), estimated at 80 to 90%, which is consistent with the ddPCR result that revealed 42% of the amplicon-containing droplets (84% of the cells) in the tumor specimen had the *PIK3CA* mutation (Figure 1C). In 2 participants (CL-U07 and CL-U13), who have levels of mutant alleles in their urine DNA that exceed our threshold for statistical significance, and in 2 participants (CL-U01 and CL-

U08), who have suspicious levels of mutant alleles in their urine DNA, we have not been able to assess these mutations in clearly affected tissue.

Discussion

Using an assay which targets 5 commonly found *PIK3CA* mutations, we confidently detected causal mutations in urine DNA samples from 6 of 17 individuals with CLOVES syndrome, including 4 of 8 individuals in whom we previously detected a mutation in affected tissue. These data indicate that screening DNA from a urine sample can find causal mutations without having to biopsy affected tissue in > 35% of individuals with CLOVES syndrome. The abundance of mutant alleles in the urine is likely to be lower than that in clinically affected tissue, since the mutant allele frequency in the urine DNA from our participants ranged from 0.25% to 7.69%, which is lower than the 1.5% to 32% range we observed when affected tissue was tested (6). Interestingly, 3 participants with detectable mutations in their urine, including one 52-year-old, have no kidney or urinary symptoms, or clinical or radiographic signs, suggesting that clinical kidney or urinary tract involvement need not be present to identify mutant alleles in urine DNA.

Four participants with CLOVES who had a known *PIK3CA* mutation in their affected tissue did not have the mutation confidently detected in urine DNA. All 4 of these participants had fewer than 2500 amplicon-containing droplets in the urine DNA sample, so a low frequency mutation could be missed or indistinguishable from a false positive result. For example, participants CL-U12 and CL-U15/16 each had mutant amplicon-containing droplets in urine DNA, but at frequencies too low to achieve statistical significance.

We employed ddPCR assays to search for 5 different mutations in urine DNA because we previously found that ~ 90% of individuals with CLOVES syndrome or KTS had one of these 5 mutations in their affected tissue (6). However, in another patient cohort, these 5 mutations accounted for only 33% of *PIK3CA* mutations found in individuals with CLOVES syndrome and 66% of *PIK3CA* mutations found in individuals with KTS (10). At present, we do not know why mutation frequencies differ between these cohorts. Given this difference between cohorts, the detection rate for the 5 *PIK3CA* mutations we sought in urine DNA may also differ. Nevertheless, since ddPCR is sensitive, specific, and inexpensive to perform, we suggest it be considered as an initial screening method for *PIK3CA* when affected tissue is unavailable. If no mutation is found, then proceeding to methods that can interrogate the entire *PIK3CA* gene such as single molecule multiplex inversion probe (smMIP) or ultradeep next-generation sequencing would be a next, but more costly, step. The same strategy can be applied if affected tissue becomes available; currently, since there are no mutation-specific targeted therapies for persons with PROS, we do not think performing ddPCR first will negatively affect clinical care by delaying the molecular diagnosis.

We did not detect mutant *PIK3CA* alleles in any of the 24 urine DNA samples from participants with KTS. Three of these individuals had *PIK3CA* mutations previously identified in their affected tissue. At present, we do not know why mutations were found in the urine from multiple persons with CLOVES and from no individual with KTS. One

possibility is in how we clinically distinguish CLOVES from KTS. Appendicular overgrowth with enlarged subcutaneous vasculature and cutaneous capillary/lymphatic malformations of the affected extremity are diagnostic criteria for KTS, which can also be seen in CLOVES syndrome; however, patients with CLOVES syndrome have additional malformations and overgrowths that can involve the torso, internal organs, and head. Thus by our definition, individuals with clinical involvement of structures outside of the appendicular skeleton are diagnosed with CLOVES and not KTS. Importantly, we did not test urine from cohorts of patients with other PROS phenotypes. In this regard, it would be interesting to screen a cohort of individuals with MCAP since their *PIK3CA* mutations appear less hypermorphic than those which cause CLOVES, thereby enabling more widespread tissue involvement (11).

Wilms tumor has been reported in 4 children and nephroblastomatosis in 2 children with *PIK3CA* mutations (3, 6, 13, 14). Clinical details have been published for several of these children and are consistent with a diagnosis of CLOVES (3, 6, 13, 15). One of these children participated in this study. Her Wilms tumor was resected when she was 2-years-old and she provided a urine sample for this study when she was 4-years-old and tumor-free. It is interesting to note that she had a mutant allele frequency in her urine DNA specimen of 2.9%, which raises the question of whether higher frequencies of *PIK3CA* mutant alleles in urine DNA might correlate with the risk of developing Wilms tumor.

Finally, most Wilms tumors occur in individuals who do not have PROS, and *PIK3CA* mutations have not previously been reported in Wilms tumor samples that have been sequenced (cancer.sanger.ac.uk). Finding a *PIK3CA* mutation in a Wilms tumor from a patient with CLOVES suggests the *PIK3CA* mutation predisposes nephroblasts to malignant transformation in a cell autonomous manner rather than a non-*PIK3CA* mutant cell becoming cancerous due to paracrine signaling from a mutant cell. Current recommendations are for children with CLOVES syndrome to be screened for Wilms tumor at regular intervals (13), although this is subject to debate (14). Screening is not currently recommended for children with KTS; however, it is unclear whether children with KTS are also at increased risk for developing Wilms tumor. A 2003 review of 115 patients followed for KTS at Boston Children's Hospital revealed no cases of Wilms tumor (24). That same study interrogated the National Wilms Tumor Study Group database and found no patients with Wilms tumor reported as also having KTS (24). There are two case reports of Wilms tumor occurring in patients diagnosed with KTS (17, 18); however, each report was published before CLOVES syndrome was described (22), which in retrospect seems the more likely diagnosis in each patient.

Conclusions

PIK3CA mutations were confidently detected in the urine DNA of > 35% of patients with CLOVES syndrome. Since urine DNA can be collected non-invasively, it may be preferable in patients with CLOVES syndrome to screen a urine DNA sample for *PIK3CA* mutations before collecting and testing affected tissue. *PIK3CA* mutant cells were present in a Wilms tumor from one individual with CLOVES syndrome, implying that *PIK3CA* mutations can predispose nephroblasts to becoming cancerous; although, experimental confirmation of this

hypothesis is needed. These data support current recommendations that children with PROS and a high likelihood of having *PIK3CA* mutant cells in the kidney undergo regular screening for Wilms tumor.

Acknowledgments

This work was supported by NIH grant AR064231 and the EXROP program of the Howard Hughes Medical Institute. The authors thank the CLOVES Family Community, the K-T Support Group, the Vascular Anomalies Center at Boston Children's Hospital, The Society for the Relief of Disabled Children, and Dr. H.P.W. Kozakewich for making this study possible.

References

1. Keppler-Noreuil KM, Rios JJ, Parker VE, et al. *PIK3CA*-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *American journal of medical genetics Part A*. 2015; 167A:287–295. [PubMed: 25557259]
2. Keppler-Noreuil KM, Parker VE, Darling TN, et al. Somatic overgrowth disorders of the PI3K/AKT/mTOR pathway & therapeutic strategies. *American journal of medical genetics Part C, Seminars in medical genetics*. 2016; 172:402–421.
3. Kurek KC, Luks VL, Ayturk UM, et al. Somatic mosaic activating mutations in *PIK3CA* cause CLOVES syndrome. *American journal of human genetics*. 2012; 90:1108–1115. [PubMed: 22658544]
4. Lindhurst MJ, Parker VE, Payne F, et al. Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in *PIK3CA*. *Nature genetics*. 2012; 44:928–933. [PubMed: 22729222]
5. Riviere JB, Mirzaa GM, O'Roak BJ, et al. De novo germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes. *Nature genetics*. 2012; 44:934–940. [PubMed: 22729224]
6. Luks VL, Kamitaki N, Vivero MP, et al. Lymphatic and other vascular malformative/overgrowth disorders are caused by somatic mutations in *PIK3CA*. *The Journal of pediatrics*. 2015; 166:1048–1054. e1041–1045. [PubMed: 25681199]
7. Osborn AJ, Dickie P, Neilson DE, et al. Activating *PIK3CA* alleles and lymphangiogenic phenotype of lymphatic endothelial cells isolated from lymphatic malformations. *Human molecular genetics*. 2015; 24:926–938. [PubMed: 25292196]
8. Rios JJ, Paria N, Burns DK, et al. Somatic gain-of-function mutations in *PIK3CA* in patients with macrodactyly. *Human molecular genetics*. 2013; 22:444–451. [PubMed: 23100325]
9. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nature genetics*. 2012; 44:941–945. [PubMed: 22729223]
10. Kuentz P, St-Onge J, Duffourd Y, et al. Molecular diagnosis of *PIK3CA*-related overgrowth spectrum (PROS) in 162 patients and recommendations for genetic testing. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2017
11. Mirzaa G, Timms AE, Conti V, et al. *PIK3CA*-associated developmental disorders exhibit distinct classes of mutations with variable expression and tissue distribution. *JCI insight*. 2016:1.
12. Forbes SA, Beare D, Gunasekaran P, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic acids research*. 2015; 43:D805–811. [PubMed: 25355519]
13. Gripp KW, Baker L, Kandula V, et al. Nephroblastomatosis or Wilms tumor in a fourth patient with a somatic *PIK3CA* mutation. *American journal of medical genetics Part A*. 2016; 170:2559–2569. [PubMed: 27191687]
14. Postema FAM, Hopman SMJ, Deardorff MA, et al. Correspondence to Gripp et al. nephroblastomatosis or Wilms tumor in a fourth patient with a somatic *PIK3CA* mutation. *American journal of medical genetics Part A*. 2017; 173:2293–2295. [PubMed: 28544518]

15. Huchtagowder V, Shenoy A, Corliss M, et al. Utility of clinical high-depth next generation sequencing for somatic variant detection in the PIK3CA-related overgrowth spectrum. *Clinical genetics*. 2017; 91:79–85. [PubMed: 27307077]
16. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, et al. Clinical delineation and natural history of the PIK3CA-related overgrowth spectrum. *American journal of medical genetics Part A*. 2014; 164A: 1713–1733. [PubMed: 24782230]
17. Ehrich JH, Ostertag H, Flatz S, et al. Bilateral Wilms's tumour in Klippel-Trenaunay syndrome. *Archives of disease in childhood*. 1979; 54:405.
18. Mankad VN, Gray GF Jr, Miller DR. Bilateral nephroblastomatosis and Klippel Trenaunay syndrome. *Cancer*. 1974; 33:1462–1467. [PubMed: 4362958]
19. Lapunzina P, Gairi A, Delicado A, et al. Macrocephaly-cutis marmorata telangiectatica congenita: report of six new patients and a review. *American journal of medical genetics Part A*. 2004; 130A: 45–51. [PubMed: 15368495]
20. Wright DR, Frieden IJ, Orlow SJ, et al. The misnomer “macrocephaly-cutis marmorata telangiectatica congenita syndrome”: report of 12 new cases and support for revising the name to macrocephaly-capillary malformations. *Archives of dermatology*. 2009; 145:287–293. [PubMed: 19289759]
21. Alomari AI. Characterization of a distinct syndrome that associates complex truncal overgrowth, vascular, and acral anomalies: a descriptive study of 18 cases of CLOVES syndrome. *Clinical dysmorphology*. 2009; 18:1–7. [PubMed: 19011570]
22. Sapp JC, Turner JT, van de Kamp JM, et al. Newly delineated syndrome of congenital lipomatous overgrowth, vascular malformations, and epidermal nevi (CLOVE syndrome) in seven patients. *American journal of medical genetics Part A*. 2007; 143A:2944–2958. [PubMed: 17963221]
23. Yeung KS, Ip JJ, Chow CP, et al. Somatic PIK3CA mutations in seven patients with PIK3CA-related overgrowth spectrum. *American journal of medical genetics Part A*. 2017; 173:978–984. [PubMed: 28328134]
24. Greene AK, Kieran M, Burrows PE, et al. Wilms tumor screening is unnecessary in Klippel-Trenaunay syndrome. *Pediatrics*. 2004; 113:e326–329. [PubMed: 15060262]

Figure 1. Causal somatic mutations in urine DNA from persons with the CLOVES subgroup of the PIK3CA-related overgrowth spectrum

Study participant with CLOVES syndrome who had a Wilms tumor. A) Photograph when 18 months old (This individual is coded as CL-U09 in the present study and as CL-2 in Kurek et al., 2012) (3). B) A 200× magnification H&E stained cryostat section of the Wilms tumor, which had been treated with chemotherapy prior to surgical excision, showing epithelial differentiation with tubular profiles. C) Dot plot graphing fluorescence intensities of individual droplets for the p.H1047R ddPCR assay performed using DNA from the Wilms tumor. Blue dots, green dots, and black dots indicate droplets containing mutant amplicon, wild-type amplicon, and no amplicon, respectively. Some droplets containing mutant and wild-type amplicon are present in the right upper quadrant. Note, 12,682 droplets contain no amplicon; of the 1119 total amplicon-containing droplets, 474 contain mutant amplicon, consistent with ~ 84% of the cells within the tumor having a *PIK3CA* mutation.

Table

Overview of ddPCR assay results for participants with CLOVES and Klippel-Trenaunay syndromes. Mutant alleles detected in the urine DNA that likely represent true positive results are highlighted in **bold**. Participants with *PIK3CA* mutations previously identified using affected tissue DNA are indicated (*), as are the ages when participants with CLOVES syndrome provided urine samples. Clinical signs and imaging-based findings are noted for individuals who had documented kidney/urinary system involvement. The amino acid designation for the mutation detected in a participant’s affected tissue and/or urine DNA is provided, along with ddPCR assay results indicating the total number of amplicon-containing droplets, the number of mutant amplicon-containing droplets, the percentage of amplicon-containing droplets with mutant alleles, the total number of droplets (amplicon-containing or not), and the *p* value that the observed result is a false positive. For the 4 participants in whom a mutation was present in affected tissue DNA and not confidently detected in the urine DNA specimen, the total numbers of ddPCR droplets, amplicon-containing droplets, and mutant amplicon-containing droplets evaluated in the urine DNA sample are provided. Since 5 different ddPCR assays were performed on each participant’s urine sample, the mean number of amplicon-containing droplets for all 5 assays is provided. Since no KTS urine sample contained 3 mutant amplicon-containing droplets, only the mean number of amplicon-containing droplets for all 5 assays is provided. Single urine samples were collected from most participants. Participants whose ddPCR assays failed for unknown reasons are indicated (#).

CLOVES Cohort										Klippel-Trenaunay Cohort			
Participant	Age year	Renal/Urinary involvement	PIK3CA Mutation	Total droplets	Total Amplicons	Mutant Amplicons	% Mutant	<i>p</i> value	Mean Total Amplicons	Participant	Mean Total Amplicons	Participant	Mean Total Amplicons
CL-U01	0.6	Hydronephrosis Renal asymmetry	E542K	52,324	2362	11	0.38	0.0013	1,784	KT-U01	2413	KT-U15	2098
CL-U02	11								5,856	KT-U02	2421	KT-U16	2922
CL-U03	1.8	Renal asymmetry							1,220	KT-U03	4539	KT-U17#	8
CL-U04	7								702	KT-U04	2209	KT-U18	739.8
CL-U05#	4.5	Scattered renal cortico-medullary echogenic foci							2	KT-U05	2592	KT-U19	5278
CL-U06	14								18,661	KT-U06	3037	KT-U20	2067
CL-U07	52		H1047R	38,306	6003	26	0.43	<0.00001	4,066	KT-U07	2874	KT-U21#	12
CL-U08	19	Hematuria Renal asymmetry	H1047R	36,806	6114	7	0.11	0.0143	5,925	KT-U08	7870	KT-U22*	1418
CL-U09*	4	Renal asymmetry Wilms tumor	H1047R	39,211	1744	51	2.92	<0.00001	1,755	KT-U09	2319	KT-U23*	3095
CL-U10*	20		E545K		2031	0			1,453	KT-U10#	20	KT-U24*	1289
CL-U11*	13		H1047L	19,578	3187	8	0.25	<0.0001	2,832	KT-U11	613	KT-U22*	1418
CL-U12*	19	Renal cysts and asymmetry	E542K	18,596	1903	2	0.03	0.2270	1,769	KT-U12	1675	KT-U23*	3095
CL-U13	14		E545K	26,003	2511	15	0.60	<0.00001	1,780	KT-U13	4551	KT-U24*	1289
CL-U14*	14	Venous malform-ation in bladder	E542K	17,698	3558	11	0.30	<0.0001	3,592	KT-U14	3981		
CL-U15/16*	2		H1047R	59,566	1421	1	0.08	0.9555	1138				
CL-U17*	6	Renal asymmetry with cortical thickening	E542K	28,174	1574	121	7.69	<0.00001	851				

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

CLOVES Cohort										Klippel-Trenaunay Cohort			
Participant	Age year	Renal/Urinary involvement	PIK3CA Mutation	Total droplets	Total Amplimers	Mutant Amplimers	% Mutant	p value	Mean Total Amplimers	Participant	Mean Total Amplimers	Participant	Mean Total Amplimers
CL-U18*	10		H1047R		1368	0			1489				