Received: 11 April 2019 | Revised: 19 August 2019 | Accepted: 5 September 2019

DOI: 10.1002/ajmg.c.31744

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART C Seminars in Medical Genetics WILEY

A dyadic genotype–phenotype approach to diagnostic criteria for Proteus syndrome

Julie C. Sapp[1] | Anna Buser[1] | Jasmine Burton-Akright[1] | Kim M. Keppler-Noreuil[1,2] | Leslie G. Biesecker[1]

[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland

[2]Division of Genetics, Children's National Medical Center, Washington, DC

Correspondence
Leslie G. Biesecker, Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, 50 South Drive Room 5140, Bethesda, MD 20892.
Email: lesb@mail.nih.gov

Funding information
National Human Genome Research Institute, Grant/Award Number: HG200388

Abstract

Phenotype-based diagnostic criteria were developed for Proteus syndrome in 1999 and updated in 2006. Subsequently, the causative mosaic gene alteration was discovered, the c.49G>A p.E17K variant in *AKT1*. As well, a number of overlapping overgrowth disorders attributable to mosaic *PIK3CA* variants have now been characterized, leading to the designation of *PIK3CA*-related overgrowth spectrum (PROS). Finally, ongoing work to better characterize Proteus syndrome has led to identification of additional features of that disorder that could be useful in diagnostic criteria. We have taken the opportunity of these discoveries to re-evaluate the Proteus syndrome diagnostic criteria. Here we propose a new set of diagnostic criteria that establishes a weighted, point-based system for the phenotypic attributes and then integrates that with the potential molecular test results to result in one of two designations: *AKT1*-related Proteus syndrome or *AKT1*-related overgrowth spectrum. A patient whose only manifestation is an *AKT1* c.49G>A-positive tumor would receive neither of these designations. Here we review the rational basis of diagnostic criteria and argue that a unitary diagnostic entity is a distinct gene-phenotype dyad and that this should be the model for all mendelian disorders. The gene-alone or phenotype-alone approach is inadequate to rigorously delineate a unitary diagnostic entity.

1 | INTRODUCTION

One of the central challenges of clinical genetics is the discovery, delineation, and taxonomy of diagnostic entities. Clinical geneticists, especially dysmorphologists, have wrestled with this, even before the word dysmorphology was applied to that clinical and scientific enterprise. Delineating disorders associated with germline DNA variants is challenging, but mosaic disorders are much more challenging. Proteus syndrome is a prototypic mosaic disorder (Biesecker, 2005; Biesecker & Sapp, 1993). In 1999 a set of clinical diagnostic criteria for Proteus were promulgated and subsequently updated and refined (Biesecker, 2006; Biesecker et al., 1999). The subsequent delineation of the cause of Proteus syndrome (Lindhurst et al., 2011) raises questions regarding how molecular testing results should be incorporated into the diagnostic criteria. Here we set out to reevaluate those clinical criteria, devise an updated, semiquantitative approach to the diagnostic criteria, and introduce the concept of a combined clinical spectrum and specific diagnosis approach to the challenges of defining diagnostic entities.

2 | METHODS

This study was performed under human subjects research protocol 94-HG-0132. All participants (or their parents, if minors) provided informed consent. We reviewed the NIH research files and NIH Clinical Center electronic health records of 65 individuals in the NIH Proteus cohort who have been shown to have a mosaic pathogenic variant in *AKT1*. We reviewed the same records for 10 individuals with relatively

severe manifestations of PROS with a confirmed mosaic variant in *PIK3CA*, reasoning that these are the patients most likely to be confused with Proteus syndrome. We tabulated the phenotypic findings and then added a weight to the scoring for these attributes. Most of these criteria are used unmodified from prior clinical diagnostic criteria (Biesecker, 2006; Biesecker et al., 1999), but several additional factors were added. We added deep vein thrombosis and pulmonary embolism as that is very common in Proteus syndrome (Keppler-Noreuil et al., 2017) and we added testicular cystadenomas/adenocarcinomas as an additional tumor phenotype as these have been identified in nearly 1/3 of males with Proteus whose genitourinary manifestations were reviewed (Keppler et al, unpublished data). We also added meningiomas to the tumor list as we have now recognized these to be common in Proteus (Keppler-Noreuil et al., 2016) and rare in PROS. Finally, we added cardiac septal lipoma as one of the options for scoring "lipomas/dysregulated adipose" (Hannoush et al., 2015). We have recognized that patients with PROS commonly have substantial congenital (prenatal) extra-CNS overgrowth (both Proteus and PROS can have hemimegancephaly). This PROS overgrowth typically includes modestly progressive, but substantial, soft tissue overgrowth which has been described as "ballooning," comprising soft tissue fullness (Turner et al., 2004). These were added as negative factors as they were recognized as common in patients with CLOVES and uncommon in Proteus. See Table 1 for details. We then adjusted these criteria until we felt that they best distinguished Proteus from CLOVES. We then set thresholds for the point scores, based on our clinical judgment, to identify the patients who we felt had such convincing manifestations (e.g., point tally) that we would clinically diagnose them with Proteus syndrome even without the confirming *AKT1* variant.

3 | RESULTS

A total of 75 individuals were evaluated in this exercise (Supplemental Table I). We developed a heuristic weighting of the criteria as described in the methods. Individuals with the *AKT1* pathogenic variant had a total score that ranged from 0 to 26 (the range of possible scores is −10 to 28, Figure 1). Several of the individuals with low scores had limited clinical data and thus the scores may have been artificially low due to absence of information. Individuals that had a clinical diagnosis of CLOVES had scores that ranged from −3 to 6. We then set a boundary at ≥15 points to consider an individual to have a clinical diagnosis of Proteus syndrome in the absence of an AKT1 variant. We would assign a diagnosis of *AKT1*-related Proteus syndrome to an individual who had a score of ≥10 points and a pathogenic mosaic variant in *AKT1*. Individuals with a score of 2 to 9 points who have a pathogenic mosaic variant in *AKT1* should be considered to have *AKT1*-related overgrowth spectrum (AROS).

4 | DISCUSSION

All pleiotropic disorders have phenotypic overlap and it can be challenging to create clinical diagnostic criteria that accurately distinguish

TABLE 1 General Criteria All the following: mosaic distribution of lesions, sporadic occurrence, and progressive course

Positive clinical criteria
Cerebriform connective tissue nevus (5 points)
Asymmetric, disproportionate overgrowth (one or more) (5 points)
(a) Limbs or
(b) Hyperostosis of the skull or
(c) Hyperostosis of the external auditory canal or
(d) Megaspondylodysplasia, scoliosis, or rib hyperostosis
Organ/visceral overgrowth (two or more) (5 points)
(a) Central nervous system or
(b) Urogenital system or
(c) Eye or
(d) Spleen or
(e) Kidney or
(f) Liver or
(g) Tonsils or adenoids or
(h) Gingiva or tongue
Bullae or cysts of the lungs (2 points)
Dysregulated adipose tissue (one or more) (2 points)
(a) Lipomas or
(b) Lipodystrophy or
(c) Myocardial septal lipoma
Linear verrucous epidermal nevus (2 points)
Vascular malformations (one or more) (2 points)
(a) Capillary malformation or
(b) Venous malformation or
(c) Lymphatic malformation
Specific tumors (1 point)
(a) Female genitourinary cystadenoma (<11 yo) or
(b) Parotid monomorphic adenoma (<11 yo) or
(c) Meningioma (meningothelial and transitional subtype) or
(d) Testicular cystadenomas or cystadenocarcinomas
Facial phenotype (three or more features) (2 points)
(a) Dolichocephaly
(b) Long face
(c) Down slanting palpebral fissures and/or minor ptosis
(d) Low nasal bridge
(e) Wide or anteverted nares
(f) Open mouth at rest
Deep vein thrombosis and/or pulmonary embolism (2 points)
Negative clinical criteria
Substantial prenatal extracranial overgrowth (−5 points)
Ballooning overgrowth (−5 points)

Proteus syndrome: a score of 10 or more points with a mosaic *AKT1* variant or 15 or more points with or without an *AKT1* mosaic variant. *AKT1*-related overgrowth spectrum: a score of 2–9 with an *AKT1* mosaic variant.

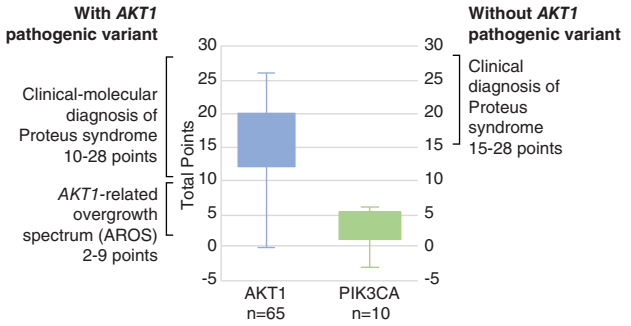

FIGURE 1 Distribution of total scores for the 75 evaluated individuals. Individuals with confirmed *AKT1* pathogenic variants would be diagnosed according to the criteria on the left side of the figure whereas individuals without an *AKT1* pathogenic variant would be diagnosed according to the criteria on the right-hand panel. Note that individuals without an *AKT1* variant with scores below 15 are not assigned one of these diagnostic descriptors. Individuals with an *AKT1* pathogenic variant and a score below 2 are not assigned one of these diagnostic descriptors

them. There is also an "elephant in the room," which is that the diagnostic criteria need to be anchored, whenever possible, and as faithfully as possible, to an unambiguous clinical truth, reality, or validity. Not doing so allows such criteria to comprise a kind of circular clinical reasoning. A simplified version of this process is a stepwise approach of (a) recognize amongst a heterogeneous group of patients a distinctive or distinguishable subset who have a similar pattern of malformation or manifestations, (b) codify and name that combination of findings as a syndrome, and then (c) say that if a patient has that combination of findings he (or she) has that syndrome, and if they do not, they do not. This approach is exemplified by the classic textbook originally written by David W Smith "*Recognizable Patterns of Human Malformation*" (Jones et al., 2013). Given the potential for circular reasoning, one can ask the question as to whether the entities described in that book and through that approach to diagnosis are in fact unitary, distinct, diagnostic entities.

David Smith and his contemporaries, the pioneers in syndrome delineation, had few tools to determine the etiologic basis of the clinical diagnostic entities that they described (B.D. Hall and J.C. Carey, personal communications). At that time, a recognizable pattern of anomalies was considered a unitary diagnostic entity and because there were few alternatives, external validation was not a significant consideration for most entities. In this way of thinking, Pallister–Hall syndrome (PHS) overlaps with Greig Cephalopolysyndactyly syndrome (GCPS) in that both can have postaxial polydactyly. However, they are mostly distinct and rarely confused. That both are caused by variants in the same gene (*GLI3*) is irrelevant—they would comprise two distinct, unitary phenotypic entities in the framework of the Smith textbook. In that same textbook, Bardet–Biedl syndrome (BBS) was considered a single distinct, unitary diagnostic entity. At that time there was no knowledge of the fact that BBS has a remarkable degree of genetic heterogeneity. It is now harder to know if BBS should be considered a unitary diagnostic entity or dozens. This can become very challenging with some disorders. The question of whether Cornelia de Lange syndrome (CdLS) is one, or instead several, unitary entities is unclear. The CdLS phenotype has been associated with pathogenic variants in both *SMC1A* and *NIPBL*. There are substantial data that the phenotypic spectrum of the patients with variants in these two genes is not the same. Thus, they may be better considered as two distinct, unitary disorders (the first is *SMC1A*-related CdLS and the second is *NIPBL*-related CdLS) and named accordingly.

These observations belie an assumption that the etiologic factor (in this case the mutated gene) has a bearing on the determination of whether a clinical diagnostic entity is unitary. This has been addressed previously in a commentary on diagnosis, in which Nat Robin and one of us (LGB) proposed integrating phenotypic, molecular, and environmental factors to designate a diagnostic entity (Robin & Biesecker, 2001). This proposal gained no traction and was probably just too cumbersome to be clinically useful. The authors of *GeneReviews* have taken a similar, but simpler and more elegant approach that we have adopted here. Entities in *GeneReviews* are sometimes referred to as "*(gene)*-related (*phenotype*)." In this way the *GeneReviews* authors are cleverly but subtly making a critical assertion—that a unitary, distinct diagnostic entity is a dyadic pair of gene and phenotype. This is advantageous compared to the previous multiaxis approach in that it is simpler, as it ignores environmental factors that were included in the multiaxis approach, as they have a modest (if any) effect on what are typically high-penetrance, single gene disorders. We propose here to generally accept the *GeneReviews* approach—we define a unitary, distinct diagnostic entity as a distinct gene-phenotype dyad. It is the combination that is unitary. One unitary entity would be *GLI3*-related PHS. Another would be *GLI3*-related Greig cephalopolysyndactyly syndrome. One would be *BBS1*-related BBS. Another would be *MKKS*-related BBS. Another would be *MKKS*-related McKusick-Kaufman syndrome. This addresses the challenge that there is not a simple one-to-one mapping of genes to phenotypes. More than one clinically distinct phenotype can map to a single gene and one phenotype can map to multiple genes.

All of the above discussion considered only constitutional genetic disorders. Mosaic pleiotropic disorders are much more challenging because differences in variant allele fraction (VAF, defined as the fraction of DNA molecules in the sample with the variant) amongst the affected tissues within an individual add another dimension of variability to this already difficult problem. One can concede that the original Proteus clinical diagnostic criteria (Biesecker, 2006; Biesecker et al., 1999) had the attribute of circular reasoning—we could recognize it clinically; therefore it was distinct. We argued, to substantial resistance, that the patients who did not meet those criteria had a different, distinct, diagnostic entity of somatic overgrowth. The delineation of the molecular basis of several disorders of somatic overgrowth provided an opportunity for external validation of these clinical criteria. We have evaluated several hundred individuals with somatic overgrowth that did not meet the Proteus syndrome criteria, and none have had the c.49G>A *AKT1* variant (Lindhurst et al., 2011; Lindhurst et al., unpublished data). Nearly all that did meet the criteria did have that *AKT1* variant. The other individuals were primarily found to have *PIK3CA* variants (Keppler-Noreuil

et al., 2014). If one accepts the gene-phenotype dyad as truth, then we have achieved external validation of the clinical criteria.

In the case of distinguishing Proteus syndrome overgrowth from non-Proteus syndrome overgrowth (CLOVES, fibroadipose overgrowth, hemihyperplasia with multiple lipomatosis, etc.) this worked well, but the mosaicism raises another challenge. In patients with Proteus syndrome, the VAF ranges from undetectable to nearly 0.50; however no known individual has a whole body burden anywhere close to 0.50, presumably because that is embryonic lethal, per the Happle hypothesis (Happle, 1986, 1987). In a single patient for whom multiple sampling was done at autopsy (Doucet et al., 2016), the variation in VAF was wide, from undetectable to 0.50 in apparently affected tissues. We have also described a minimally affected individual who has Proteus manifestations only of his lower legs and low VAF (Wee et al., 2014). An argument of *reductio ad absurdum* is useful here. As we have shown highly variable, and sometimes low, VAF in patients, we must assume that other patients exist with yet subtler manifestations and more delimited mosaicism and/or low VAF. Mosaic *PIK3CA* variants have been shown to exist in only a finger or toe. This begs the question of what must be the lower limit of mosaic distribution and VAF—which is unanswerable. Indeed, we hypothesize that some individual may have but a single cell with the c.49G>A *AKT1* variant. This clearly cannot be considered *AKT1*-related Proteus syndrome as the phenotype half of the dyad is absent. However, there is another domain of c.49G>A *AKT1* variants which include a number of malignancies including meningiomas, breast, endometrium, and others (COSMIC data, https://cancer.sanger.ac.uk/cosmic, accessed Dec 20, 2018). Interestingly, the meningothelial and transitional subtypes of meningiomas with the higher fraction of *AKT1 variants* share features with the meningiomas observed in patients with Proteus syndrome, and they are one of the most common tumors associated with that syndrome (Keppler-Noreuil et al., 2016). Therefore, one could posit that a sporadic meningothelial meningioma with the c.49G>A *AKT1* variant and no other manifestations of Proteus syndrome is a highly delimited, mild form of Proteus syndrome. The clinical geneticist and clinical oncologist would likely balk at this—to consider this to be Proteus syndrome may be a bridge too far. In the scoring system proposed here, this individual would be assigned one point (specific tumor). If they had no other manifestations of overgrowth, but did have the *AKT1* c.49G>A variant in their tumor, they would not meet the threshold for *AKT1*-Related overgrowth spectrum, which assumes that most geneticists would not consider this to be Proteus syndrome. Some may feel that Proteus is an inappropriate diagnostic descriptor for many individuals at the lower end of this range. Yet in rejecting the diagnosis of Proteus syndrome for a patient with an isolated meningioma, one recognizes the impossibility of a rational, objective lower boundary of what Proteus syndrome (or any mosaic disorder) can be defined to be. This cannot be anything but an arbitrary diagnostic heuristic. We accept the heuristic nature of this boundary and propose a practical solution. For practical considerations, we assert that Proteus syndrome comprises a constellation of recognizable features associated with the c.49G>A *AKT1* variant and define both phenotypic and VAF thresholds below which a patient does not have Proteus syndrome but instead has an entity that we will term "*AKT1*-related overgrowth spectrum" (AROS). At the same time, we recognize that there is no rational, objective, biological division between the lower end of the Proteus syndrome designation and the upper end of AROS. Alternatively, one could concede the entire spectrum to AROS, or one could designate patients with the recognizable syndrome as "AROS-Proteus syndrome subtype." We have also adjusted the thresholds so that an individual with a Proteus syndrome-related tumor and no other manifestations of Proteus syndrome would not meet the threshold for AROS (and certainly not Proteus syndrome)—again because we assume that most clinicians would prefer to diagnose that patient with, simply, "meningioma." Similarly, in the proposed scoring system, individuals must have two or more organ/visceral overgrowths in order to obtain the points for this criterion. This is to prevent a person with isolated hepatomegaly or another common, enlarged organ from meeting the threshold of AROS.

The merit of the dichotomizing approach of defining both Proteus syndrome and AROS is that it preserves the clinical syndromic label that clinicians and affected individuals and families have found to be valuable, meaningful, and useful. It also captures the biologic reality of AKT1 as the driver of the pathophysiology and recognizes the fundamental issue that this is a spectrum with presumably continuous variation. It also recognizes what we have observed when following the history of non-Proteus syndrome overgrowth. We first fissioned Proteus syndrome into "true" Proteus syndrome and entities that were not Proteus syndrome. In the latter, entities were recognized such as hemihyperplasia with multiple lipomatosis (HHML) (Biesecker et al., 1998), CLOVES syndrome (Sapp et al., 2007), fibroadipose overgrowth (Lindhurst et al., 2012), isolated macrodactyly (Rios et al., 2013), and many other descriptors. We subsequently unified these into the umbrella term of "*PIK3CA*-Related Overgrowth Spectrum" or PROS (Keppler-Noreuil et al., 2015). However, our informal clinical observations of our colleagues and patients do not suggest wide uptake of this unification. Indeed, patients and doctors very much seem to like descriptors such as CLOVES syndrome, when the individual has all (or most) of the clinical features (as opposed to just diagnosing them with PROS). Our inclination is to be respectful of this preference and as in our AKT1 proposal above, recognize both the spectrum and the specifics. That is to say, an individual who had all six of the cardinal manifestations of CLOVES could still be diagnosed with CLOVES or "PROS-CLOVES type." A patient with only two or three manifestations of CLOVES would be better diagnosed with simply, PROS. A patient with an isolated, overgrown foot should also be designated as PROS. Ideally, this would be coupled to the molecular half of the dyad, such as "PIK3CA-related CLOVES syndrome." PROS has the dyad concept (gene-related phenotype) already incorporated.

By stratifying individuals based on phenotype, we recognize that mosaic disorders must be considered as a continuum, but that heuristic thresholds for the syndromic designations in diagnostic schema are practical, useful, and necessary. We also recognize that molecular genetic testing has a critical role. Importantly, we do not mean to suggest that individuals with a score above 15 should not be tested—the diagnosis is a devastating one—the clinician should be as certain as possible that the diagnosis is correct. Such testing will likely also be

essential for selecting targeted treatments. However, before such testing results may be available for an individual, a firm clinical diagnosis can be useful and important. In the larger picture, the cost of an *AKT1* variant detection will be a trivial contributor to the medical costs incurred by nearly any individual with this disease and should be performed in all individuals who have manifestations compatible with this disorder.

This dyadic approach to diagnosis of mosaic overgrowth disorders recognizes fundamental attributes of the affected individual including the phenotypic manifestations and the pathogenic gene variant, both of which are critical considerations for diagnosis. We have endeavored to incrementally improve previous clinical diagnostic heuristics with this dyadic approach to both increase the accuracy and ease of clinical diagnosis. To this end we have created a clinical worksheet that practitioners can use to score individuals in the clinic (Supplemental Information). Accurate diagnosis is challenging for all ultra-rare disorders, more so for mosaic disorders. We hope that this approach addresses that challenge.

ACKNOWLEDGEMENTS

The authors thank John C. Carey and Bryan D. Hall for critical evaluation of an earlier draft of parts of this manuscript. The opinions expressed here are those of the authors. This research was supported by the Intramural Research Program of the National Human Genome Research Institute grant HG200388 05. LGB is a member of the Illumina medical ethics board, receives royalties from Genentech, Inc., and in-kind research support from ArQule, Inc. LGB receives honoraria from Cold Spring Harbor Press.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The primary data are not publicly available due to privacy or ethics restrictions.

ORCID

Kim M. Keppler-Noreuil https://orcid.org/0000-0001-8250-164X
Leslie G. Biesecker https://orcid.org/0000-0002-0197-3811

REFERENCES

Biesecker, L. G. (2005). Proteus syndrome. In S. B. Cassidy & J. E. Allanson (Eds.), *Management of genetic syndromes* (pp. 449–456). New York: Wiley.

Biesecker, L. G. (2006). The challenges of Proteus syndrome: Diagnosis and management. *European Journal of Human Genetics*, *14*, 1151–1157.

Biesecker, L. G., Happle, R., Mulliken, J. B., Weksberg, R., Graham, J. M., Jr., Viljoen, D. L., & Cohen, M. M., Jr. (1999). Proteus syndrome: Diagnostic criteria, differential diagnosis, and patient evaluation. *American Journal of Medical Genetics*, *84*, 389–395.

Biesecker, L. G., Peters, K. F., Darling, T. N., Choyke, P., Hill, S., Schimke, N., … Cohen, M. M., Jr. (1998). Clinical differentiation between Proteus syndrome and hemihyperplasia: Description of a distinct form of hemihyperplasia. *American Journal of Medical Genetics*, *79*, 311–318.

Biesecker, L. G., & Sapp, J. C. (1993). Proteus Syndrome. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews*. Seattle, WA: University of Washington.

Doucet, M. E., Bloomhardt, H. M., Moroz, K., Lindhurst, M. J., & Biesecker, L. G. (2016). Lack of mutation-histopathology correlation in a patient with Proteus syndrome. *American Journal of Medical Genetics. Part A*, *170*, 1422–1432.

Hannoush, H., Sachdev, V., Brofferio, A., Arai, A. E., LaRocca, G., Sapp, J., … Keppler-Noreuil, K. M. (2015). Myocardial fat overgrowth in Proteus syndrome. *American Journal of Medical Genetics Part A*, *167A*, 103–110.

Happle, R. (1986). Cutaneous manifestation of lethal genes. *Human Genetics*, *72*, 280.

Happle, R. (1987). Lethal genes surviving by mosaicism: A possible explanation for sporadic birth defects involving the skin. *Journal of the American Academy of Dermatology*, *16*, 899–906.

Jones, K. L., Jones, M. C., & Campo, M. d. (2013). *Smith's recognizable patterns of human malformation* (7th ed.). Philadelphia, PA: Elsevier Saunders.

Keppler-Noreuil, K. M., Baker, E. H., Sapp, J. C., Lindhurst, M. J., & Biesecker, L. G. (2016). Somatic AKT1 mutations cause meningiomas colocalizing with a characteristic pattern of cranial hyperostosis. *American Journal of Medical Genetics. Part A*, *170*, 2605–2610.

Keppler-Noreuil, K. M., Lozier, J. N., Sapp, J. C., & Biesecker, L. G. (2017). Characterization of thrombosis in patients with Proteus syndrome. *American Journal of Medical Genetics. Part A*, *173*, 2359–2365.

Keppler-Noreuil, K. M., Rios, J. J., Parker, V. E., Semple, R. K., Lindhurst, M. J., Sapp, J. C., … Biesecker, L. G. (2015). *PIK3CA*-related overgrowth spectrum (PROS): Diagnostic and testing eligibility criteria, differential diagnosis and evaluation. *American Journal of Medical Genetics. Part A*, *167A*, 287–295.

Keppler-Noreuil, K. M., Sapp, J. C., Lindhurst, M. J., Parker, V. E., Blumhorst, C., Darling, T., … Biesecker, L. G. (2014). Clinical delineation and natural history of the PIK3CA-related overgrowth spectrum. *American Journal of Medical Genetics. Part A*, *164A*, 1713–1733.

Lindhurst, M. J., Parker, V. E., Payne, F., Sapp, J. C., Rudge, S., Harris, J., … Semple, R. K. (2012). Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in *PIK3CA*. *Nature Genetics*, *44*, 928–933.

Lindhurst, M. J., Sapp, J. C., Teer, J. K., Johnston, J. J., Finn, E. M., Peters, K., … Biesecker, L. G. (2011). A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *New England Journal of Medicine*, *365*, 611–619.

Rios, J. J., Paria, N., Burns, D. K., Israel, B. A., Cornelia, R., Wise, C. A., & Ezaki, M. (2013). Somatic gain-of-function mutations in *PIK3CA* in patients with macrodactyly. *Human Molecular Genetics*, *22*, 444–451.

Robin, N. H., & Biesecker, L. G. (2001). Considerations for a multiaxis nomenclature system for medical genetics. *Genetics in Medicine*, *3*, 290–293.

Sapp, J. C., Turner, J. T., van de Kamp, J. M., van Dijk, F. S., Lowry, R. B., & Biesecker, L. G. (2007). Newly delineated syndrome of congenital lipomatous overgrowth, vascular malformations, and epidermal nevi (CLOVE syndrome) in seven patients. *American Journal of Medical Genetics Part A*, *143A*, 2944–2958.

Turner, J. T., Cohen, M. M., Jr., & Biesecker, L. G. (2004). Reassessment of the Proteus syndrome literature: Application of diagnostic criteria to published cases. *American Journal of Medical Genetics. Part A*, *130A*, 111–122.

Wee, J. S., Mortimer, P. S., Lindhurst, M. J., Chong, H., Biesecker, L. G., & Holden, C. A. (2014). A limited form of proteus syndrome with bilateral plantar cerebriform collagenomas and varicose veins secondary to a mosaic AKT1 mutation. *JAMA Dermatology*, *150*, 990–993.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Sapp JC, Buser A, Burton-Akright J, Keppler-Noreuil KM, Biesecker LG. A dyadic genotype–phenotype approach to diagnostic criteria for Proteus syndrome. *Am J Med Genet Part C*. 2019;181C:565–570. https://doi.org/10.1002/ajmg.c.31744