Clinical Study (Prospective, Retrospective, Case Series)

OPEN

Journal of Vascular Anomalies
An open access multidisciplinary journal
ISSVA

Parkes Weber Syndrome

Contribution of the Genotype to the Diagnosis

Themis-Areti A. Andreoti[a,b], Aleksandra Tuleja[b,c], Yvonne Döring[c,d,e,f], Massimo Maiolo[c,g,h], André Schaller[i], Erik Vassella[g], Christiane Zweier[i], Laurence M. Boon[j,k], Miikka Vikkula[j,k,l], Jochen Rössler[a,m,*], Sarah M. Bernhard[c,d,*], Iris Baumgartner[c,d,*]

Abstract

Objectives: Parkes Weber syndrome (PWS) is a rare disorder that combines overgrowth, capillary malformations, and arteriovenous malformations (AVM)/arteriovenous fistulas, for which underlying activating mutations in the ras/mitogen-activated protein kinase/extracellular-signal-regulated kinase signaling pathway have been described. The clinical overlap with Klippel-Trenauny syndrome, associated with mutations in *PIK3CA*, is significant. This case series aimed to elaborate on the phenotypic description of PWS, to underline its clinical overlap with Klippel-Trenauny syndrome and nonsyndromic AVM, and to evaluate the contribution of genotypic characterization to the diagnosis.

Methods: All patients diagnosed with PWS upon enrollment in the Bernese VAScular COngenital Malformations (VASCOM) cohort were included. The diagnostic criteria of PWS were retrospectively reviewed. A next-generation sequencing (NGS) gene panel (TSO500, Illumina) was used on tissue biopsy samples.

Results: Overall, 10/559 patients of the VAScular COngenital Malformations cohort were initially diagnosed with PWS. Three patients were reclassified as nonsyndromic AVM (Kristen Rat Sarcoma Viral oncogene homolog [*KRAS*], *KRAS*+tumor protein p53, and protein tyrosine phosphatase non-receptor type 11). Finally, 7 patients fulfilled all clinical diagnostic criteria of PWS. Genetic testing was available in 5 PWS patients. Only 1 patient had the classic *RASA1* mutation; another patient had mutations in G protein subunit alpha q (*GNAQ*) and phosphatase and tensin homolog. In a third case, a *PIK3CA* mutation was detected. In 2 patients, no mutations were identified.

Conclusion: Overgrowth syndromes with vascular malformations are rare and their clinical overlap hampers the classification of individual phenotypes under specific syndrome labels, sometimes even despite genetic testing. To provide optimal patient care, an accurate phenotypic description combined with the identification of molecular targets for precision medicine may be more meaningful than the syndrome classification itself.

Keywords: genetic testing, Klippel-Trenauny Syndrome, overgrowth syndromes, Parkes Weber Syndrome, PIK3CA-related overgrowth spectrum, *PIK3CA*

[a]*Division of Pediatric Hematology and Oncology, Department of Pediatrics, Inselspital-University Hospital of Bern, University of Bern, Bern, Switzerland;* [b]*Graduate School for Health Sciences, University of Bern, Bern, Switzerland;* [c]*Division of Angiology, Swiss Cardiovascular Center, Inselspital-University Hospital of Bern, University of Bern, Bern, Switzerland;* [d]*Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland;* [e]*Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University Munich (LMU), Munich, Germany;* [f]*DZHK (German Centre for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany;* [g]*Institute of Tissue Medicine and Pathology, University of Bern, Bern, Switzerland;* [h]*SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland;* [i]*Department of Human Genetics, Inselspital-University Hospital of Bern, University of Bern, Bern, Switzerland;* [j]*Center for Vascular Anomalies, Cliniques Universitaires Saint-Luc, Brussels, Belgium;* [k]*VASCERN (European Network of Rare Vascular Diseases) Reference Center, VASCA working group;* [l]*Department of Human Genetics, Laboratory of Human Molecular Genetics, de Duve Institute, Catholic University of Louvain, Brussels, Belgium;* [m]*Department of Vascular Medicine, National Reference Centre for Rare Lymphatic and Vascular Diseases, UA11 INSERM–UM IDESP, Campus Santé, Montpellier Cedex, France*

**Drs Rössler, Bernhard, and Baumgartner contributed equally.*

Funding/Support: I.B. (Principle Investigator), J.R (Co-Investigator), T.A.A. (PhD student), A.T. (PhD student), M.M. (Scientist), L.B. (Co-Investigator) and M.V. (Co-Investigator) were supported by the Swiss National Research Foundation, Sinergia grant CRSII5_193694 2021-2024.

Financial Disclosure: Andreoti, Bernhard, Tuleja, Baumgartner, and Rössler are investigators in the EPIK-P2 study (https://clinicaltrials.gov/ct2/show/NCT04589650), sponsored by Novartis. Rössler is currently an employee of Novartis Pharma Basel. The rest of the authors have no conflicts of interest with regard to the content of this report.

Authorship confirmation

T.A.A.: *Conceptualization, methodology, writing (original draft, lead).* **A.T.:** *Resources, investigation.* **Y.D.:** *Resources, investigation.* **M.M.:** *Resources.* **A.S.:** *Resources.* **E.V.:** *Resources.* **C.Z.:** *Writing (review and editing, supporting).* **L.B.:** *Writing (review and editing, supporting).* **M.V.:** *Writing (review and editing, supporting).* **J.R.:** *Project supervision, funding acquisition, writing (review and editing, lead).* **S.B.:** *Project supervision, resources, investigation, writing (original draft, supporting).* **I.B.:** *Project supervision, funding acquisition, writing (review and editing, supporting).*

Previous Presentations: This article is an original work that has neither been published nor is under consideration for publication elsewhere. Preliminary data were presented at the VAC 2023–International Vascular Anomalies Conference, January 31–February 03, 2023, Brussels, Belgium, https://vacure-conference.net/ (poster presentation).

Correspondence: Jochen Rössler, MD, Division of Pediatric Hematology and Oncology, Department of Pediatrics, University Hospital of Bern, University of Bern, Freiburgstrasse 15, CH-3010 Bern, Switzerland (jochen.roessler@insel.ch).

Journal of Vascular Anomalies (2023) 4:e076

Received: 18 May 2023; Accepted 24 October 2023

Published online 13 November 2023

DOI: 10.1097/JOVA.0000000000000076

Introduction

Various rare, heterogenous conditions with significant clinical overlap, that present with congenital vascular malformations and segmental overgrowth, are categorized among "vascular malformations associated with other anomalies" in the current International Society for the Study of Vascular Anomalies classification.[1] The continually evolving advances in the realm of molecular genetic pathology have revealed activating mutations in the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and ras/mitogen-activated protein kinase/extracellular-signal-regulated kinase (RAS/MAPK/ERK) signaling pathways as the genetic background for the majority of vascular malformations, and this subcategory is no exception.[2,3]

In Parkes Weber syndrome (PWS), overgrowth and capillary malformations (CM) are combined with fast-flow vascular malformations (arteriovenous malformation [AVM] and arteriovenous fistula [AVF]).[4] Both germline and somatic activating mutations in various components of the RAS/MAPK/ERK pathway have been identified in patients with PWS in the last few years.[5–7] In patients with AVM, when not all phenotypic characteristics of the syndrome are present, the diagnosis of nonsyndromic AVM is to be considered.

Often enough, PWS is misdiagnosed as Klippel-Trenauny syndrome (KTS), which combines overgrowth and CM with slow-flow vascular malformations (venous malformation [VM] ± lymphatic malformation [LM]) of an extremity.[8] KTS is the most important representative of the *PIK3CA*-related overgrowth spectrum (PROS)[9] and of the entire subcategory of vascular malformations with overgrowth.

Objectives

The focus of this case series remains primarily on the phenotypic description of PWS, with an emphasis on the clinical overlap with PROS/KTS as well as with nonsyndromic AVM. The consequent challenges in differential diagnosis are underlined, and the possible contribution of genetic testing to the diagnosis is evaluated.

Methods

Ethical approval of the cohort and informed consent forms

The Bernese VAScular COngenital Malformation (VASCOM) cohort of the Inselspital-University Hospital of Bern, Switzerland (ethics board number 2017-01960, Ethics Committee of the Canton of Bern), has been enrolling consecutive patients with congenital extra-cranial/extra-spinal vascular malformations since 2008.[10] In our center, genetic testing was implemented as a standard of care in October 2020. All patients (or their legal guardians) provided written informed consent forms for data analysis and genetic testing.

Study sample, clinical data collection, and genetic testing

Patient data were prospectively collected using caregiver-completed electronic case-report forms. All patients that were recruited to the VASCOM cohort between 2008 and 2021 were reviewed for eligibility; patients diagnosed with PWS at the time of recruitment were included in this case series.

In the second step, the diagnostic criteria for PWS were retrospectively reviewed in all patients:

1. AVM/AVF, confirmed by digital subtraction angiography and/or magnetic resonance angiography (MRA), with net shunt volume measurement using duplex ultrasound;
2. clinically diagnosed cutaneous CM; and
3. signs of overgrowth: soft-tissue hypertrophy, confirmed on MR, and limb length difference (LLD), objectively assessed by clinical measurement and/or long-leg radiography.

With the intention of underscoring the extent of diagnostic challenges, we did not exclude patients with a retrospective diagnosis revision.

Genetic testing was performed by next-generation sequencing (NGS) on frozen tissue available from diagnostic biopsies using the TruSight Oncology 500 gene panel (TSO500, Illumina);[11] the TSO500 was originally designed to target exonic regions of 523 genes associated with solid tumors and covers most of the pathogenic variants that cause vascular malformations. Sequencing was performed at the Clinical Genomics Lab of the Inselspital. The term "mutation" is used throughout this article in place of the more precise term "pathogenic variant." Variants of unknown significance are not presented.

Results

Study sample description

A total number of 559 pediatric and adult patients with non-central nervous system vascular malformations were enrolled in the VASCOM cohort,[10] and tissue biopsy samples of 90 patients (16.1%) were genotyped until June 2022.

Upon enrollment in the VASCOM cohort, 10 out of 559 (1.8%) patients were clinically diagnosed with PWS. In 3 out of 10 patients, we retrospectively revised the diagnosis to nonsyndromic AVM, combined with either overgrowth or CM (Kristen Rat Sarcoma Viral oncogene homolog [*KRAS*], case 8; *KRAS*+ tumor protein p53 [*TP53*], case 9; protein tyrosine phosphatase non-receptor type 11 [*PTPN11*], case 10). Seven patients (1.25%) fulfilled all clinical diagnostic criteria of PWS; 2 patients had negative genetic testing (cases 2 and 6), whereas somatic mutations were found in 3 patients (*RASA1*, case 4; G protein subunit alpha q [*GNAQ*] + phosphatase and tensin homolog [PTEN], case 5; *PIK3CA*, case 7). Genetic testing was not available in 2 PWS patients (cases 1 and 3) (Figure 1 and Table 1).

Case 1: Parkes Weber syndrome, no genetic testing

An athletic 22-year-old woman with AVM of the right arm, cutaneous CM, and LLD first noticed a difference in arm circumference at 17 years of age. A net shunt volume of 2080 mL/min in the right subclavian artery was stable over time, with normal left ventricular (LV) function on the echocardiogram. Diffuse multifocal arteriovenous (AV)-shunts were revealed on angiography so that an endovascular embolization had no expected benefit; the patient was treated conservatively with supportive measures (Figure 2).

Case 2: Parkes Weber syndrome, no mutation detected

A 30-year-old man presented with a microfistular AVM infiltrating muscles and bones of the left pelvis and leg, LLD, lymphedema, and elevated D-dimers. A shunt in the common femoral artery was progressive (3 L/min at age 30 years, 9 L/min at age 44 years), leading to secondary LV hypertrophy,

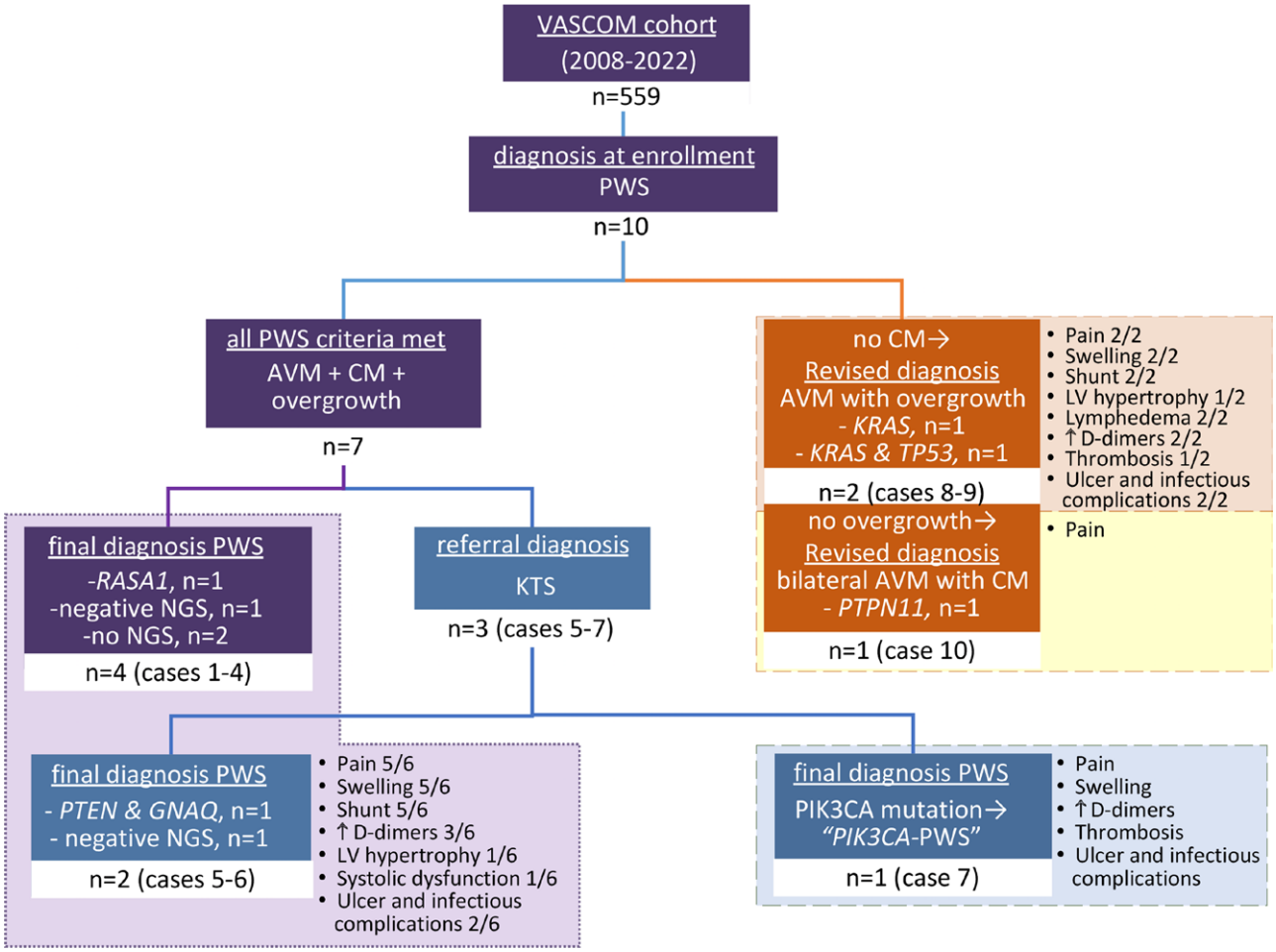

Figure 1. Flowchart of the diagnostic procedure.

yet with a preserved ejection fraction (EF). The patient underwent multiple embolizations; a probatory off-label treatment with mTOR inhibitor (sirolimus) was ineffective and, thus, interrupted after 1 year. The patient was later genotyped, and no relevant mutation was identified (Figure 2).

Case 3: Parkes Weber syndrome, no genetic testing

A 28-year-old man with a history of a ruptured aneurysm of the left external iliac artery and a lower leg amputation in the context of PWS (skin CM, AVM, history of LLD) was referred to our center. The patient was on thalidomide and underwent multiple coil- and alcohol embolizations for the bone-infiltrating AVM of the residual limb. After a second amputation over the knee, his clinical course was complicated with chronic nonphantom-limb pain, as well as poor wound healing, progressive ulceration, skin necrosis, chronic wound infection, and recurrent bleeding with transfusion-requiring iron-deficiency anemia. The patient was transferred to a palliative care setting, where he died at age 30 due to major bleeding.

Case 4: Parkes Weber syndrome, RASA1 mutation

A 40-year-old woman with skin CM, microfistular AVM, overgrowth of the right lower limb, and mildly reduced EF (56%), was referred to us for further treatment. She had a previous history of epiphysiodesis in childhood and coiling of various microfistular AV-shunts in puberty. After an uncomplicated clinical course for almost 2 decades under compression therapy, she presented with recurrent episodes of debilitating pain at age 34 years and was treated with a debulking operation; this was preferred to embolization due to her microfistular angiographic findings. After short-term postoperative relief, the symptoms partially recurred.

A markedly increased shunt volume with partial arterialization of dilated superficial veins was seen on the duplex ultrasound, which reduced from 3.5 to 1.5 L/min after alcohol embolization. She was not restricted in her daily physical activities, and her cardiac function was normal. A classic *RASA1* mutation was detected (Figure 2).

Case 5: Parkes Weber syndrome, mutations in GNAQ and PTEN

A 55-year-old man with a previous KTS diagnosis (cutaneous CM, LM, overgrowth of the left leg, elevated D-dimers) and worsening symptoms after significant weight gain, attributed to decreased physical activity during the COVID-19 pandemic, was referred to us for further treatment. He had a previous history of stripping of a lateral marginal vein (LMV), was currently under compression therapy and lymph drainages, and had a clinical course complicated with recurrent foot ulcers, erysipelas, and sepsis.

The duplex ultrasound showed an increased flow in the left common femoral artery, with a net shunt volume of 200 mL/min; after the refusal of the patient to undergo an MRA, a diagnostic angiography confirmed the presence

Table 1.

Overview of the Case Series

Patient Characteristics			Clinical Manifestations, Complications, and Treatments								Biopsy and Genetic Testing*		
			Vascular Malformations (and Anomalies)			Signs of Overgrowth							
Case	Age, Sex	Localization (Tissue Compartment)	AVM/ AVF	Skin CM	Other	STH	Bone OG/LLD	Symptoms and Complications	Treatment	Gene	Mutation	VAF %
1	22, f	Right arm (SC)	AVM†	+	No	+	Yes	Pain, swelling Shunt 2 L/min	-	N/A	(Not genotyped)	N/A
2	30, m	Left leg and pelvis (SC, M, B)	AVM‡	+	PFO Intestinal vascular malformations	+	Yes	Pain, swelling, ↑D-dimers 752–7315 mg/L Lymphedema Shunt 3–> 9 L/min LV hypertrophy, Biatrial and RV dilatation	Debulking surgery, alcohol embolizations, sclerotherapy, sirolimus.	-	None detected	-
3	28, m	Left leg and pelvis (SC, M, B)	AVM†	+	No	+	Yes; history	Pain, swelling Rupture of external iliac artery aneurysm Ulcer, necrosis Wound infection, sepsis	Debulking surgery, alcohol and coil embolizations, thalidomide, lower and upper leg amputation.	N/A	(Not genotyped)	N/A
4	40, f	Right leg (SC, M)	AVM‡	+	-	+	Yes; corrected	Pain, swelling Shunt 3.5–>1.5 L/min EF 56%	Epiphysiodesis, debulking surgery, alcohol and coil embolizations.	RASA1	p.P886Afs*12 (c.2654dupG)	6.2%
5	55, m	Left leg and foot (NA)[34]	AVM‡	+	(Varicose veins)	+	Yes	Pain, ↑D-dimers 808–1830 mg/L Ulcer Erysipelas, sepsis Shunt 0.2 L/min	Stripping of marginal vein	GNAQ PTEN	p.Gly48Val (c.143G>T) p.G1u242Ter (c.724G>T)	11.1% 2.2%
6	20, f	Right leg and foot (SC)	AVM‡	+	Marginal vein	+	Yes	Swelling Shunt 0.5 L/min	Sclerotherapy	-	None detected	-
7	39, f	Left leg and foot (SC, M)	AVM‡	+	(Varicose veins)	+	Macrodactyly digiti pedis II&III; corrected	Pain, swelling, ↑D-dimers 523–1091 mg/L Thrombosis Ulcer Erysipelas No significant shunt	Ligation of feeder arteries, alcohol embolizations, sclerotherapy, stripping of varicose veins, toe amputation.	PIK3CA	p.Arg108_Ile112del(c.323_337del) Revised diagnosis: "Phenotype: PWS/ Genotype: PIK3CA"	10.5%

(Continued)

Table 1.
(Continued)

Patient Characteristics			Clinical Manifestations, Complications, and Treatments								Biopsy and Genetic Testing*		
			Vascular Malformations (and Anomalies)			Signs of Overgrowth							
Case	Age, Sex	Localization (Tissue Compartment)	AVM/ AVF	Skin CM	Other	STH	Bone OG/LLD	Symptoms and Complications	Treatment	Gene	Mutation	VAF %
8	29, f	Right leg (SC, M)	AVM†	No	Marfan's syndrome with aortic aneurysm &insufficiency	+	Yes; overcorrected	Pain, swelling, ↑D-dimers 989–10132 mg/L Lymphedema Cutaneous hyperpigmentation¶ Ulcer Erysipelas Shunt 2.8 L/min	epiphysiodesis, ligation of the right internal iliac artery, multiple debulking operations, alcohol and coil embolizations, sclerotherapy, thalidomide, trametinib.	KRAS	p.Gly12Asp (c.35G>A) Revised diagnosis: "non-syndromic AVM with overgrowth"	12.4%
9	61, m	Right leg and foot (SC)	AVM†	No	(Varicose veins), tibiofibular trunk aneurysm	+	Yes; corrected	Pain, swelling, ↑D-dimers 1080–2088 mg/L Thrombosis Lymphedema Ulcer Erysipelas Shunt 1.5–2 L/min LV hypertrophy	Epiphysiodesis, alcohol embolizations, stripping of varicose veins.	KRAS TP53	p.Gly12Asp (c.35G>A) p.Arg175His (c.524G>A) Revised diagnosis: "non-syndromic AVM with overgrowth"	1.8% 1.8%
10	34, m	Left and right lower leg (SC, B)	AVM‡	+	VSD (bilateral varicose veins)	No	No	Pain No significant shunt	Debulking surgery, coil embolizations, stripping of varicose veins.	PTPN11	p.Ser502Pro (c.1504T>C) Revised diagnosis: "non-syndromic AVM with CM"	5.1%

Age at referral/enrollment is presented.

B, bones/joints; GNAQ, G protein subunit alpha q; KRAS, Kristen Rat Sarcoma Viral oncogene homolog; LLD, limb length difference, m/f, male/female; M, muscle; NA, not available; OG, overgrowth; PFO, patent foramen ovale; PTEN, phosphatase and tensin homolog; PTPN, protein tyrosine phosphatase non-receptor type 11; SC, subcutaneous; STH, soft-tissue hypertrophy; TP53, tumor protein p53; VAF, variant allele frequency; VSD, ventricular septal defect.

*All variants presented were classified as likely pathogenic (TIER 2); variants of unknown significance are not presented. Based on the low VAF rates, all the variants identified presumably correspond to somatic mutations.

†Patients 1, 3, 8, and 9 had AVM with macro- and microfistular components. (AVM type II and IV).

‡Patients 2, 4, 5, 6, 7, and 10 had a microfistular subtype of AVM, with post-capillary (capillary-venule, CV) microfistular shunts (CV-AVM).[12]

§The tissue compartments involved are not available, since this patient refused MRA.

¶Skin hyperpigmentation due to chronically elevated hydrostatic pressure was differentiated from CM.

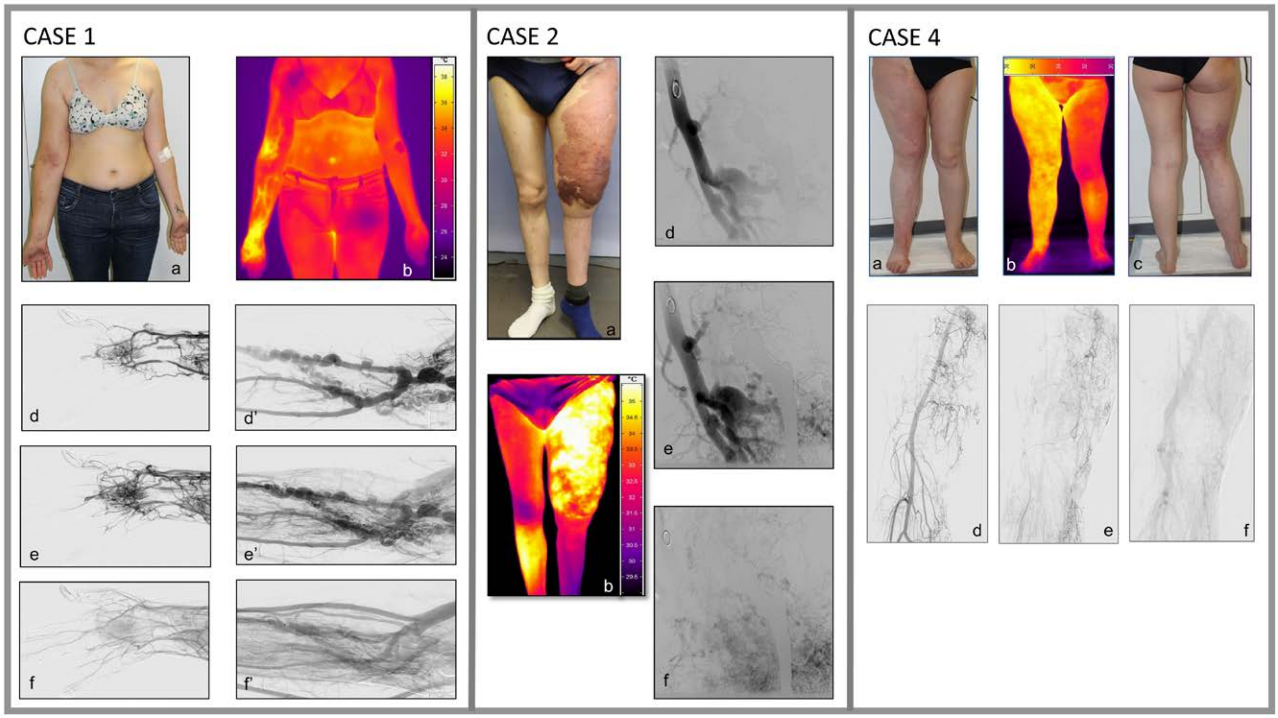

Figure 2. Patients with final diagnosis of PWS (cases 1, 2, and 4*). Patient photos, thermographic images (A–C), and digital subtraction angiography (DSA) at an early (D), late (E), and very late phase (F). * Case 3 was referred to our center after amputation of the affected limb, which is why no adequate imaging is available.

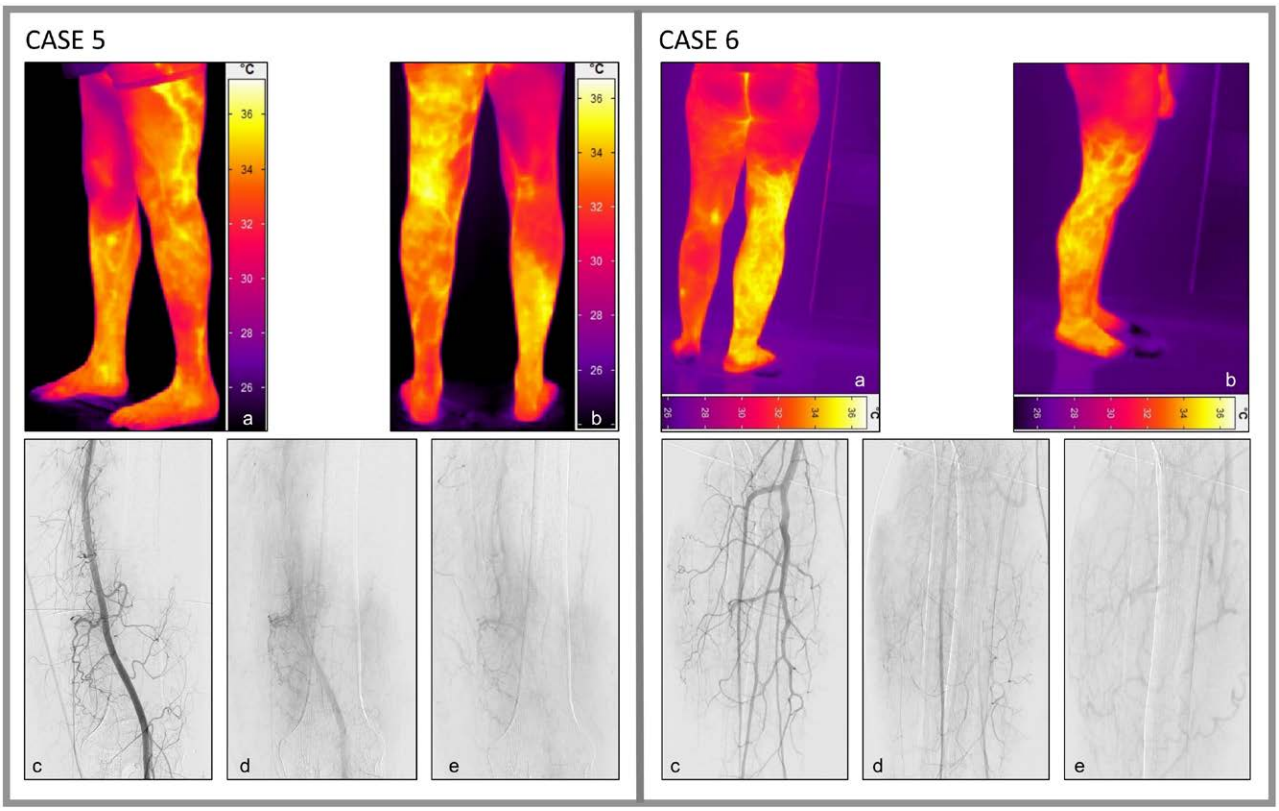

Figure 3. Patients with final diagnosis of PWS and referral diagnosis of KTS (cases 5 and 6). Patient photos, thermographic images (A–D), and digital subtraction angiography (DSA) at an early (E), late (F), and very late phase (G) of cases 5 and 6 are shown. KTS indicates Klippel-Trenauny syndrome; PWS, Parkes Weber syndrome.

of microfistular AVMs, and his former KTS diagnosis was revised to PWS. The TSO500 NGS panel showed mutations in *GNAQ* and *PTEN* (Figure 3).

Case 6: Parkes Weber syndrome, no mutation detected

A 20-year-old woman formerly diagnosed with KTS (cutaneous CM of the right leg, LMV, overgrowth) was referred to our clinic for a preoperative evaluation before an orthopedic knee surgery. The LMV was successfully treated with sclerotherapy. A conspicuous dilatation of the femoral vein in the distal third of the thigh was seen on MRA, raising suspicion for an AVM. The presence of multiple microfistular shunts with the early venous filling was angiographically confirmed, and the Doppler ultrasound revealed a shunt of 500 mL/min in favor of the affected extremity, so that we eventually diagnosed her with PWS (Figure 3).

Case 7: Parkes Weber syndrome, nonhotspot PIK3CA mutation

A 39-year-old woman with a KTS diagnosis since childhood (cutaneous CM, VM, left foot overgrowth with macrodactyly, elevated D-dimers) and a previous history of toe amputation, thrombosis, and repeated stripping of recurrent varicose veins was referred to us when angiographic evidence of a fast-flow malformation was found. Multiple microfistular shunts were indeed demonstrated on MRA without significant shunt volume on duplex sonography. The diagnosis was revised to PWS, and the patient was treated with multiple embolizations. Surprisingly, the TSO500 NGS panel revealed a somatic nonhotspot *PIK3CA* mutation. Since neither PWS nor PROS could describe this patient accurately enough, we assigned a descriptive diagnosis (phenotype of PWS/ genotype *PIK3CA*) (Figure 4).

Case 8: multiple arteriovenous malformations with overgrowth, somatic KRAS mutation, and germline FBN1 mutation

We examined a 29-year-old woman with overgrowth apparent since birth, multiple AVMs in the entire right lower limb, and elevated D-dimers; compression socks were used since puberty when varicose veins first appeared. She also had lymphedema, and her clinical course was complicated by recurrent erysipelas and ulcers. Previous treatments included epiphysiodesis as well as multiple debulking operations and embolizations, after which, by the age of 33, there were no angiographic indications for further treatment.

Then, 4 years later, the patient presented symptoms of progressive exercise intolerance; a recurrence of the AVM with a shunt volume of 2.8 L/min was confirmed on tomography and duplex ultrasound. Although her systolic function was normal (EF >60%), an aortic root aneurysm and annuloaortic ectasia were seen on the echocardiogram; this raised suspicion for a concomitant Marfan syndrome, which was genetically

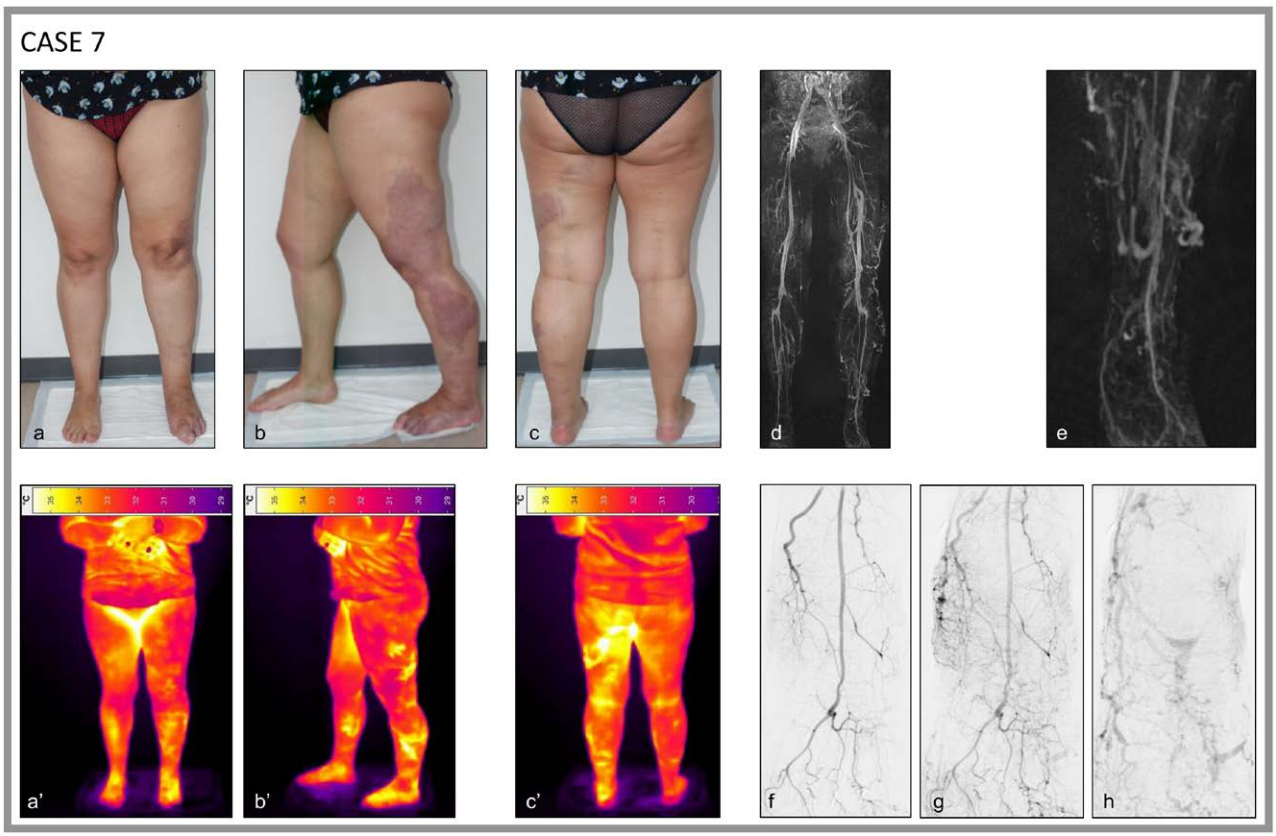

Figure 4. Patient with *PIK3CA*-PWS and referral diagnosis of KTS (case 7). Patient photos (A–C), corresponding thermographic images (A'–C'), MR images with time-resolved angiography with interleaved stochastic trajectories (TWIST) and golden-angle radial sparse parallel (GRASP) sampling sequences of both legs (D) and left calf and foot (E), as well as digital subtraction angiography (DSA) of the left calf and foot at an early (F), late (G), and very late phase (H). KWS indicates Klippel-Trenauny syndrome; PWS, Parkes Weber syndrome.

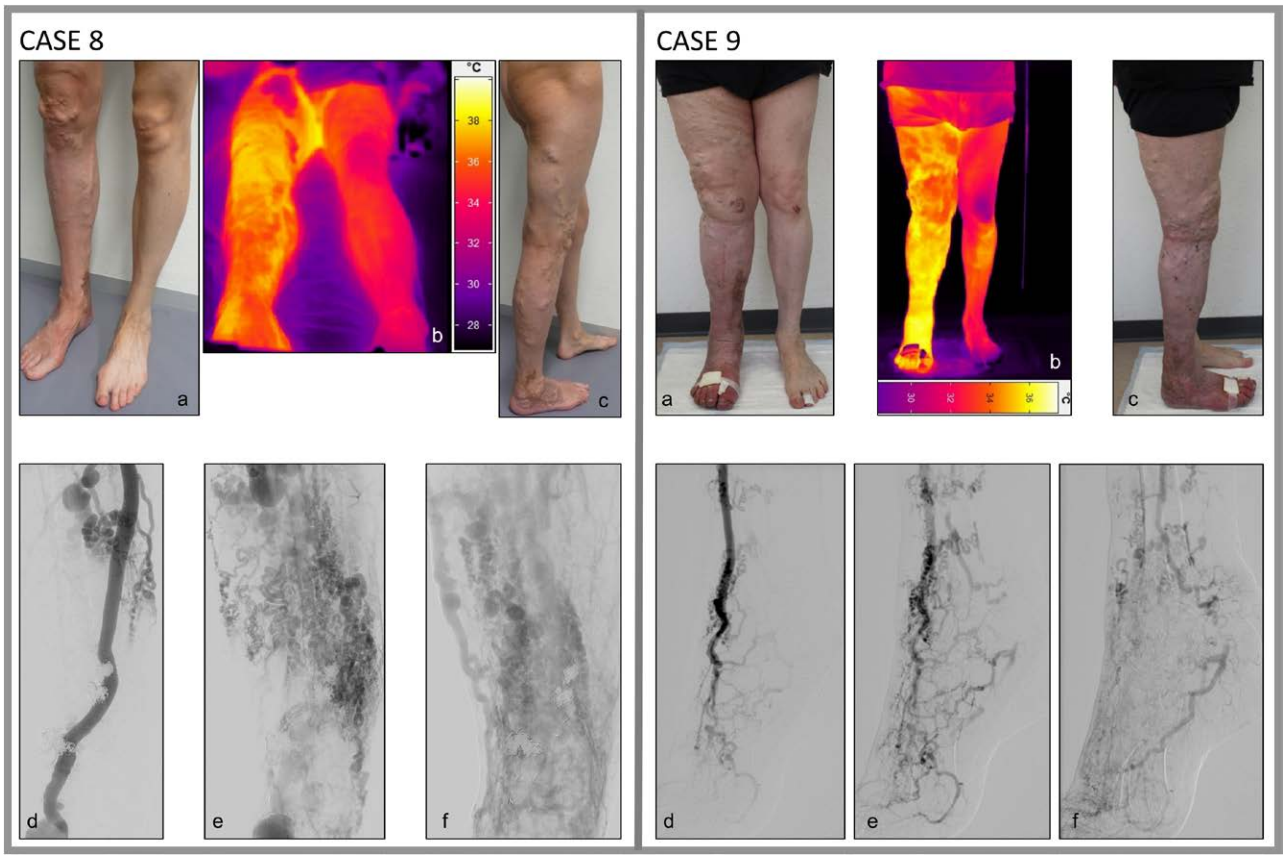

Figure 5. Patients with diagnosis revision to AVM with overgrowth (cases 8 and 9). Patient photos, thermographic images (A–C), and digital subtraction angiography (DSA) at an early (D), late (E), and very late phase (F). AVM indicates arteriovenous malformations; CM, capillary malformations

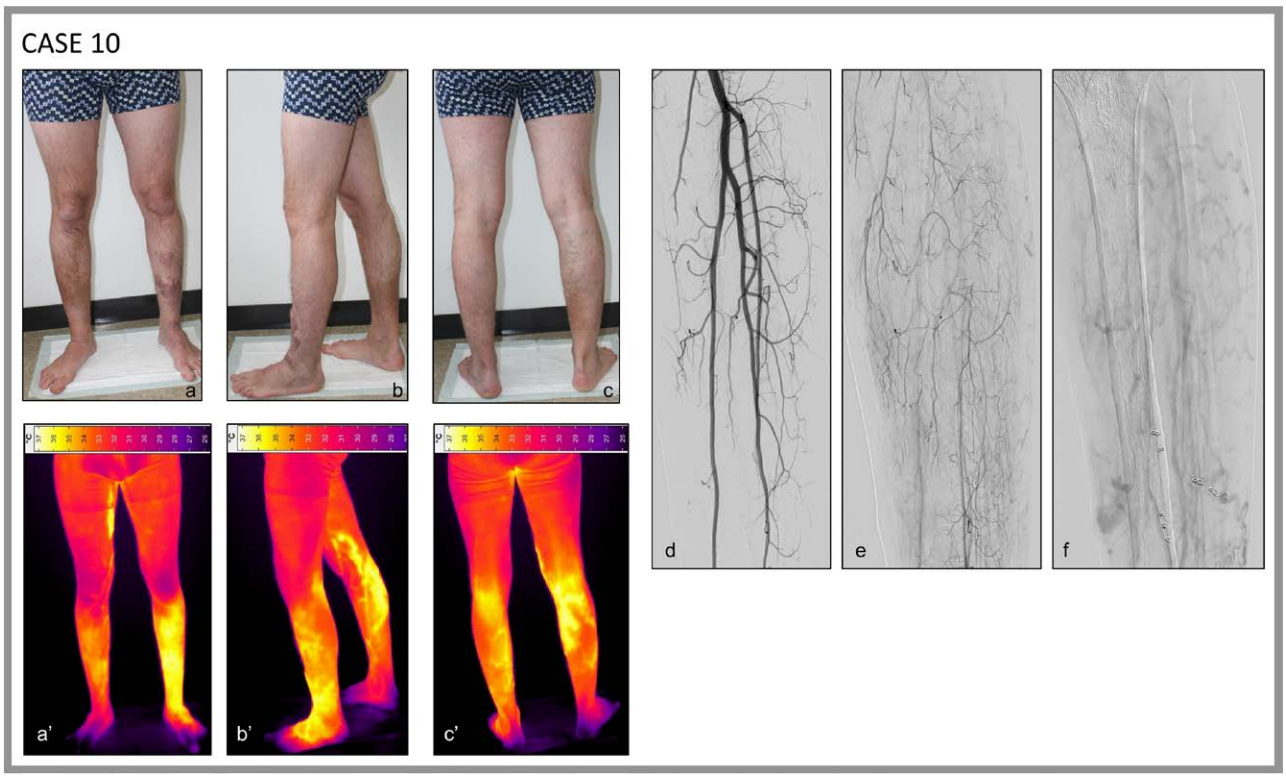

Figure 6. Patient with diagnosis revision to bilateral AVM with CM (case 10). Patient photos (A–C), corresponding thermographic images (A'–C'), and digital subtraction angiography (DSA) of the left calf at an early (D), late (E), and very late phase (F).

confirmed. A previously treated aneurysmatic internal iliac artery was retrospectively attributed to Marfan syndrome and further treated with embolizations and ligation. The patient was on thalidomide, and additional embolizations were performed to treat the AVM; a *KRAS* mutation was found in the TSO500 NGS panel, and the patient was started on trametinib.

The patient's cutaneous lesions were compatible with hyperpigmentation, secondary to elevated hydrostatic pressure, and her history was negative for birthmarks; in the absence of cutaneous CM, the diagnosis was revised to nonsyndromic multiple AVMs with overgrowth (Figure 5).

Case 9: arteriovenous malformations with overgrowth, mutations in KRAS and TP53

A 61-year-old man with AVM, overgrowth of the right lower leg and foot, and elevated D-dimers was referred to us for further treatment after vascular surgery for thrombosis of the distal superficial femoral artery and popliteal artery, secondary to an aneurysm of the tibiofibular trunk. The patient had massive varicose veins, recurrent thrombophlebitis, recurrent ulcers, and erysipelas, as well as lymphedema with lymphorrhea. Previous treatments included osteotomy (LLD + 5 cm), multiple treatments for his varicose veins, shunt ligations, and conservative measures (ongoing compression treatment and lymph drainages).

An AVM with multiple arteriovenous shunts was demonstrated on MRI, and the duplex ultrasound revealed a net shunt volume of 1500 mL/min in favor of the affected leg. The patient was treated with embolizations; postinterventionally, he had another episode of malleolar ulceration, complicated with recurrent erysipelas and impaired wound healing, requiring autologous skin grafting. The shunt volume was stable over time, and his echocardiogram showed a hypertrophic LV with preserved EF. In the absence of cutaneous CM, the diagnosis was revised to nonsyndromic AVM with overgrowth. Mutations in *KRAS* and *TP53* were identified (Figure 5).

Case 10: arteriovenous malformations and capillary malformations, PTPN11 mutation

A 34-year-old man, with a congenital CM of the left lower leg and bilateral varicose veins, was referred to our center. A surgical resection of symptomatic varicose veins was performed at age 20; intraoperatively, atypical findings were present but not further investigated. The patient's recurrent symptoms stabilized after selective micro-coiling of the feeder branches of the left tibial artery at age 25. Our imaging studies revealed microfistular AVMs in both lower extremities, with concomitant venous insufficiency of the right great saphenous vein. After two debulking operations, the patient was asymptomatic. However, a local relapse occurred, underscoring the spontaneously progressive nature of the malformation. Given the presence of a somatic *PTPN11* mutation, the therapeutic option of a mitogen-activated protein kinase kinase inhibitor (trametinib) was discussed but not favored, because of the patient's asymptomatic postoperative course and wish to procreate. In the absence of overgrowth, the initial diagnosis of PWS was revised to nonsyndromic AVM with CM (Figure 6).

Discussion

We report on 10 patients that were phenotypically diagnosed with PWS upon enrollment in our monocentric vascular malformation cohort. After reviewing the clinical findings, we assigned a definite diagnosis of PWS to only 7 patients (7/10); only 1 of them had a classic *RASA1* mutation. Interestingly, a nonhotspot *PIK3CA* mutation was identified in 1 patient with the PWS phenotype. Cases 4,5, and 7–10 have been included in other studies.[13,14]

In the systematic review of Banzic et al. reporting on 48 patients with PWS, the lower extremities were affected in 87.5% of patients.[4] In our series, all 7 of our PWS patients were unilaterally affected with AVM and cutaneous capillary malformation (ie, nevus flammeus) in 1 extremity (6/7 lower extremity) along with ipsilateral soft-tissue and skeletal overgrowth (soft-tissue hypertrophy 7/7, LLD in favor of the affected limb 7/7).

All patients underwent multiple treatments, including vascular surgery (debulking surgery: PWS 3/7 and AVM 2/3; stripping of varicose veins and/or LMV: PWS 2/7 and AVM 2/3), orthopedic surgery (epiphysiodesis: PWS 1/7 and AVM 2/3; amputation: PWS 2/7 and AVM 0/3), alcohol or coil embolization or sclerotherapy (PWS 5/7 and AVM 3/3), and medical treatment (PWS 2/7 [thalidomide, n = 1; sirolimus, n = 1] and AVM 1/3 [thalidomide and trametinib, n = 1]).

Skin ulcerations and infectious complications were common in our case series (PWS 3/7; AVM 2/3). D-dimers are reportedly normal in simple fast-flow vascular malformations, yet often elevated in complex or syndromic AVM.[14] We found elevated D-dimers >500 mg/L in 3/7 patients with PWS and in 2/3 patients with nonsyndromic AVM; 1 patient from each group had thrombotic complications (case7, *PIK3CA*-PWS; case 9, AVM with mutations in *KRAS* and *TP53*). Abnormal echocardiographic findings were found in 2 of 7 PWS patients (case 2, LV hypertrophy; case 4, systolic dysfunction with EF 56%). One patient with PWS was amputated twice and died of bleeding complications (case 3).

Parkes Weber syndrome versus Klippel-Trenauny syndrome: overlap and differences

Overall, 3 of the 7 patients with a definite PWS diagnosis had been referred to us as KTS patients. Overlapping features of PWS and KTS often make diagnosis challenging. The key feature to clinically differentiate PWS from KTS is the presence of a fast-flow vascular malformation.[15] The skin is warm, and a bruit or thrill might be present.[16] On Doppler ultrasound, arterial waveforms and high flow may be observed in venous vessels, suggesting arteriovenous shunting. Additionally, the net shunt volume and the increased arterial flow volume can be calculated in comparison to the nonaffected extremity.[17] The presence of AVM/AVF can be angiographically confirmed. In noninvasive imaging studies such as MRA, AV shunting is demonstrated by an early filling of draining veins.[18] The AVM in PWS causes secondary venous hypertension, which can lead to aneurysmal degeneration of draining veins, axial reflux, edema, and chronic inflammatory changes.[19] On the other hand, KTS is often associated with the presence of an LMV, a persistent avalvular embryonic vein that often runs underneath the cutaneous CM. Although it is often misdiagnosed as a varicose vein, the LMV is actually a congenital vascular malformation that represents the most common VM in KTS.[20,21] Even though it

is not considered as part of PWS,[15] an LMV was described in 2 of our 7 PWS patients.

It is not uncommon for patients with overgrowth to be categorized as KTS regardless of the type of underlying vascular malformation, much in a similar way that different vascular malformations are often misdiagnosed as "hemangiomas." Inversely, to the best of our knowledge, there is only 1 case report published in the literature regarding a patient with PROS who was initially misdiagnosed as PWS. Su et al.[22] reported on a patient with CM, limb overgrowth, and clinical signs typical of a fast-flow malformation who was therefore diagnosed with PWS. However, imaging studies ruled out the presence of AVM, and a *PIK3CA* mutation was detected so that a revised PROS diagnosis was determined.[22]

PWS has to be differentiated from KTS because their clinical course is different. PWS has a more aggressive clinical course, and the follow-up and treatment plan should be appropriately adjusted. In PWS, the overgrowth of the affected extremity is progressive until epiphyseal closure, while in KTS, overgrowth tends to occur in the first decade of life, with implications on the timing of epiphysiodesis.[18] Chronic or recurrent ulcers due to venous stasis may complicate either of the 2 conditions. In PWS, ulcers may also be caused due to an ischemic steal phenomenon caused by the AVMs, and patients with extended wounds are at high risk for infection and amputation.[23,24] Furthermore, patients with PWS should be monitored for symptoms of heart failure. It is known from the literature that up to one-third of patients with PWS may develop high-output heart failure as a result of hemodynamically significant arteriovenous shunting. In some cases, the amputation of the affected limb proves to be an inevitable therapeutic strategy.[4]

In contrast to PWS, KTS can usually be managed conservatively without major complications. Additionally, emerging evidence supports targeted treatment with inhibition of the PI3K/AKT/mTOR signaling pathway as a promising therapeutic strategy in patients with KTS/PROS.[21,25] The mTOR inhibition with sirolimus may modestly reduce overgrowth in KTS,[26] and PI3K inhibition with alpelisib may alleviate various symptoms in PROS.[27]

Genetic testing

Molecular pathology genetic testing of affected tissue provides clinicians with useful additional information. PWS was initially associated with *RASA1* mutations in families with an autosomal dominant inheritance pattern and a phenotype with multifocal CM and CM-AVM type 1.[5,28] On the contrary, germline *RASA1* mutations are not found in KTS.[29] Recently, a germline mutation in *EPHB4* was found in families with similar manifestations (multifocal CM and CM-AVM type 2, with or without PWS) that tested negative for the *RASA1* mutation.[30] Sporadic somatic mutations of *RASA1* have also been identified in PWS.[6,7,31] More recently, *KRAS* mutations were also reported in patients with PWS and LM.[32]

In our case series, genetic testing results were available for 8 patients (5/7 PWS and 3/3 AVM) and a clinically relevant genetic mutation was identified in 6 patients (3/7 PWS and 3/3 AVM) and concerned the RAS/MAPK/ERK pathway in 5 patients (2/7 PWS and 3/3 AVM). As reflected by the low variant allele frequency rates (Table 1), all of them were somatic mutations; besides, none of our patients had a positive family history of vascular malformations or overgrowth syndromes.

All 3 patients that were reclassified as nonsyndromic AVM had activating somatic mutations in the RAS/MAPK/ERK pathway (case 8, *KRAS* mutation; case 9, *KRAS* mutation with concomitant mutation in *TP53*; case 10, PNTP11 mutation). Pathogenic variants of *TP53* have not been linked to vascular malformations yet; further research is needed to unveil the full spectrum of mutations that cause vascular malformations.

Out of 5 genetically tested PWS patients, TSO500 NGS genetic testing was negative in 2 PWS patients (cases 2 and 6). Only 1 PWS patient had the typical *RASA1* mutation (case 4). Another PWS patient had mutations in both *GNAQ* and *PTEN* (case 5). *GNAQ* mutations have been found in patients with CM with or without overgrowth,[31,33] but there are no previous reports of *GNAQ* mutations in PWS. Loss-of-function *PTEN* mutations are believed to lead to activation of AKT in the PI3K/AKT/mTOR signaling pathway and have been associated with AVM and other vascular malformations.[3] Therefore we cannot be sure whether one of the mutations or the combination thereof was responsible for the phenotype in case 5. Interestingly, a *PIK3CA* mutation was detected in a patient with PWS (case 7).

PIK3CA mutations cause various slow-flow vascular malformations and are by definition pathognomonic of PROS, but have not yet been associated with syndromic AVM.[8,34] On the other hand, the evidence of an AVM is the key differentiating aspect of PWS.[4,15] In this series, we report on 1 patient with an unusual combination of both findings (case 7). The presence of AVM was verified by angiography, and the former clinical diagnosis of KTS was revised to PWS. When the patient was later genotyped, a *PIK3CA* mutation was detected by the TSO500 NGS panel.

This specific *PIK3CA* mutation has been reported in colorectal cancer (p.[Arg108_Ile112del], c.323_337del)[35] and categorized as likely pathogenic (TIER 2) in the human genomic variant search engine VarSome. A nearby amino-acid deletion had been previously reported to be an activating mutation;[36] we thereby presume this to be an activating mutation, too. Taking into consideration: (1) the above, (2) the fact that fast-flow vascular malformations are not part of KTS, and (3) the broad spectrum of *PIK3CA*-related disorders, we could conclude that this patient should be retrospectively classified as "unspecified PROS." Yet, an accurate phenotypic description is more important than the classification itself; since the features that determine the patient's prognosis and treatment plan are not well represented under the umbrella term of PROS, we opted for a mere description of the patient's phenotypic and genotypic findings instead, that is, "*PIK3CA*-PWS or phenotype of PWS with a *PIK3CA* mutation." In the absence of other reports, whether this case represents a random exception or is part of a distinct subgroup of PWS caused by *PIK3CA* mutations, remains unclear.

Limitations

This was a retrospective case series that was not designed to assess, and does not report on, treatment outcomes. As expected in such rare diseases, our sample size is small and did not allow for the identification of possible commonalities linked to genotypes. The fact that 3 out of 10 included patients did not fulfill all diagnostic criteria of PWS is a further limitation; we believe, however, that it is useful to present these 3 patients along with the patients with a definite PWS diagnosis, in an effort to underline the extent of the existing

clinical overlap. Genetic testing was not available for 2 of our PWS patients, who had a clinical course on either end of the spectrum; the first one had no need of treatment thanks to a very favorable clinical course so a biopsy was not indicated. The second one had a very complicated clinical course and died long before genetic testing was implemented in our center. Last but not least, the possible presence of pathogenic variants in the 2 cases with negative results in the TSO500 NGS panel cannot be excluded since whole genome or whole exome sequencing was not performed.

Conclusion

This study demonstrates the principal clinical manifestations of PWS as well as the diagnostic challenges even in specialized centers. The extent of clinical overlap between PWS and KTS is highlighted. We report a novel possible association of a nonhotspot *PIK3CA* mutation with PWS with microfistular AVM; this is a finding that needs further research and verification of its clinical relevance.

Overgrowth syndromes with vascular malformations are rare, and their overlapping phenotype makes differential diagnosis difficult. Overlapping nomenclature (ie, Klippel-Trenauny-Weber syndrome)[37,38] and inconsistent terminology add up to the difficulties imposed by their clinical overlap. The differences in phenotypic features, clinical course, genetic background, and possible targeted treatment options, underline the importance of the correct diagnosis for appropriate patient management. We thereby agree that the use of any combined terms should be discouraged.[21]

Our study suggests that genetic characterization does not necessarily facilitate the classification between overlapping clinical entities. Moreover, classifying each patient under the syndrome label that most accurately describes both their phenotypic and genotypic findings may not always be possible. Phenotypic classification is likely to remain the cornerstone of diagnosis, treatment indication, and outcome evaluation. To provide optimal patient care, an accurate phenotypic description in combination with genetic testing may be more meaningful than the syndrome classification itself; when establishing a personalized treatment plan, the indication for treatment should be based on the phenotype, and according to the genetic findings, the possibility to benefit from precision medicine should also be assessed.

Increased disease burden and the need for multiple interdisciplinary interventions underpin the need to optimize medical treatment; targeted treatment approaches are promising yet not well established. Even though NGS did not prove to be helpful as a diagnostic tool in our case series, the importance of genotyping still lies in uncovering the pathophysiology of vascular malformations and in identifying molecular targets for precision medicine. PWS may be associated with a wide spectrum of underlying mutations, not necessarily exclusively in the RAS/MAPK/ERK pathway. As genetic testing remains negative for a number of patients, the genetic variability within the PWS spectrum is to be determined.

Acknowledgements

We would like to thank our patients and their families for supporting the cohort, as well as their referring and attending physicians. We also thank Mrs. Györgyi Hamvas (study coordinator) and the rest of our study nurses for their valuable support in study administration and data monitoring.

References

1. Classification. *International Society for the Study of Vascular Anomalies*. [cited 2022 Nov 3]. Available at: https://www.issva.org/classification
2. Dekeuleneer V, Seront E, van Damme A, Boon LM, Vikkula M. Theranostic advances in vascular malformations. *J Invest Dermatol*. 2020;140(4):756–763.
3. Queisser A, Seront E, Boon LM, Vikkula M. Genetic basis and therapies for vascular anomalies. *Circ Res*. 2021;129(1):155–173.
4. Banzic I, Brankovic M, Maksimović Z, Davidović L, Marković M, Rančić Z. Parkes Weber syndrome-Diagnostic and management paradigms: a systematic review. *Phlebology*. 2017;32(6):371–383.
5. Revencu N, Boon LM, Mulliken JB, et al. Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat*. 2008;29(7):959–965.
6. Boccara O, Eyries M, Pannier S, Ariche-Maman S, Hadj-Rabia S, Coulet F. Parkes-Weber syndrome related to RASA1 mosaic mutation. *Clin Genet*. 2021;99(2):330–331.
7. Flores Daboub JA, Grimmer JF, Frigerio A, et al. Parkes Weber syndrome associated with two somatic pathogenic variants in RASA1. *Cold Spring Harb Mol Case Stud*. 2020;6(4):a005256.
8. Diociaiuti A, Paolantonio G, Zama M, et al. Vascular birthmarks as a clue for complex and syndromic vascular anomalies. *Front Pediatr*. 2021;9:730393.
9. Keppler-Noreuil KM, Rios JJ, Parker VER, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *Am J Med Genet A*. 2015;167A(2):287–295.
10. Tuleja A, Bernhard SM, Hamvas G, et al. Clinical phenotype of adolescent and adult patients with extracranial vascular malformation. *J Vasc Surg Venous Lymphat Disord*. 2023;11(23):1034–1044.e3.
11. Froyen G, Geerdens E, Berden S, Cruys B, Maes B. Diagnostic validation of a comprehensive targeted panel for broad mutational and biomarker analysis in solid tumors. *Cancers*. 2022;14(10):2457.
12. Vuillemin N, Bernhard S, Haine A, et al. Capillary-venule malformation is a microfistulous variant of arteriovenous malformation. *J Vasc Surg Venous Lymphat Disord*. 2021;9(1):220–225.
13. Bernhard SM, Tuleja A, Laine JE, et al. Clinical presentation of simple and combined or syndromic arteriovenous malformations. *J Vasc Surg Venous Lymphat Disord*. 2022;10(3):705–712.
14. Bernhard SM, Döring Y, Vassella E, Amstutz U, Zweier C, Rössler J, et al. Oncogene targeted next-generation-sequencing in extra-cranial arteriovenous malformations. 2023.
15. Cohen MM. Klippel-Trenaunay syndrome. *Am J Med Gen*. 2000;93(3):171–175.
16. Amaral JG, Lara-Corrales I. Vascular anomalies: clinical perspectives. *Pediatr Radiol*. 2022;52(2):249–261.
17. Madani H, Farrant J, Chhaya N, et al. Peripheral limb vascular malformations: an update of appropriate imaging and treatment options of a challenging condition. *Br J Radiol*. 2015;88(1047):20140406.
18. Ziyeh S, Spreer J, Rössler J, et al. Parkes Weber or Klippel-Trenaunay syndrome? Non-invasive diagnosis with MR projection angiography. *Eur Radiol*. 2004;14(11):2025–2029.
19. Nassiri N, Cirillo-Penn NC, Thomas J. Evaluation and management of congenital peripheral arteriovenous malformations. *J Vasc Surg*. 2015;62(6):1667–1676.
20. Lee BB. Marginal vein is not a varicose vein; it is a venous malformation. *Veins and Lymphatics*. 2014;3(2).
21. Ishikawa K, Yamamoto Y, Funayama E, Furukawa H, Sasaki S. Wound-Healing problems associated with combined vascular malformations in Klippel-Trenaunay syndrome. *Adv Wound Care (New Rochelle)*. 2019;8(6):246–255.
22. Su LX, Sun Y, Wang Z, et al. Complex vascular anomalies and tissue overgrowth of limbs associated with increased skin temperature and peripheral venous dilatation: Parks Weber syndrome or PROS? *Hereditas*. 2022;159(1):1.
23. Deshpande AA, Pandey NN, Singh SP, Kumar S. Klippel–Trenaunay and Parkes–Weber syndromes: differentials of congenital vascular syndromes! *Indian J Surg*. 2020;82(6):1300–1301.
24. Conway AM, Qato K, Nguyen Tran NT, et al. Embolization techniques for arteriovenous malformations in Parkes-Weber Syndrome. *Ann Vasc Surg*. 2020;69:224–231.
25. Hughes M, Hao M, Luu M. PIK3CA vascular overgrowth syndromes: an update. *Curr Opin Pediatr*. 2020;32(4):539–546.
26. Parker VER, Keppler-Noreuil KM, Faivre L, et al.; PROMISE Working Group. Safety and efficacy of low-dose sirolimus in the PIK3CA-related overgrowth spectrum. *Genet Med*. 2019;21(5):1189–1198.
27. Venot Q, Blanc T, Rabia SH, et al. Targeted therapy in patients with PIK3CA-related overgrowth syndrome. *Nature*. 2018;558(7711):540–546.

28. Eerola I, Boon LM, Mulliken JB, et al. Capillary malformation–arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet*. 2003;73(6):1240–1249.
29. Revencu N, Boon LM, Dompmartin A, et al. Germline mutations in RASA1 are not found in patients with Klippel-Trenaunay syndrome or capillary malformation with limb overgrowth. *Mol Syndromol*. 2013;4(4):173–178.
30. Amyere M, Revencu N, Helaers R, et al. Germline Loss-of-Function mutations in EPHB4 cause a second form of capillary malformation-arteriovenous malformation (CM-AVM2) deregulating RAS-MAPK signaling. *Circulation*. 2017;136(11):1037–1048.
31. Diociaiuti A, Rotunno R, Pisaneschi E, et al. Clinical and molecular spectrum of sporadic vascular malformations: a single-center study. *Biomedicines*. 2022;10(6):1460.
32. Eng W, Sudduth CL, Konczyk DJ, et al. Parkes Weber syndrome with lymphedema caused by a somatic KRAS variant. *Cold Spring Harb Mol Case Stud*. 2021;7(6):a006118.
33. Bichsel C, Bischoff J. A somatic missense mutation in GNAQ causes capillary malformation. *Curr Opin Hematol*. 2019;26(3):179–184.
34. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, et al. Clinical delineation and natural history of the PIK3CA-Related overgrowth spectrum. *Am J Med Genet A*. 2014;164A(7):1713–1733.
35. Hampel H, Pearlman R, Beightol M, et al.; Ohio Colorectal Cancer Prevention Initiative Study Group. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer. *JAMA Oncol*. 2018;4(6):806–813.
36. Ng PKS, Li J, Jeong KJ, et al. Systematic functional annotation of somatic mutations in cancer. *Cancer Cell*. 2018;33(3):450–462.e10.
37. Peña MR, Ovando E. [Klippel-Trenaunay-Weber syndrome with vesical and uterine involvement treated by endoscopic and endovascular routes]. *Medicina (B Aires)*. 2020;80(1):84–86.
38. Oda K, Morimoto D, Kim K, Yui K, Kitamura T, Morita A. Spinal cavernous angioma associated with Klippel-Trenaunay-Weber syndrome: case report and literature review. *World Neurosurg*. 2018;109:333–337.