Comprehensive targeted next-generation sequencing in patients with slow-flow vascular malformations

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Abstract

Recent studies have shown that the PI3K signaling pathway plays an important role in the pathogenesis of slow-flow vascular malformations (SFVMs). Analysis of genetic mutations has advanced our understanding of the mechanisms involved in SFVM pathogenesis and may identify new therapeutic targets. We screened for somatic variants in a cohort of patients with SFVMs using targeted next-generation sequencing. Targeted next-generation sequencing of 29 candidate genes associated with vascular anomalies or with the PI3K signaling pathway was performed on affected tissues from patients with SFVMs. Fifty-nine patients with SFVMs (venous malformations n = 21, lymphatic malformations n = 27, lymphatic venous malformations n = 1, and Klippel–Trenaunay syndrome n = 10) were included in the study. *TEK* and *PIK3CA* were the most commonly mutated genes in the study. We detected eight *TEK* pathogenic variants in 10 samples (16.9%) and three *PIK3CA* pathogenic variants in 28 samples (47.5%). We also identified a pathogenic variant in *RASA1* in one sample. In total, 38 of 59 patients (64.4%) with SFVMs harbored pathogenic variants in these three genes involved in the PI3K signaling pathway. Inhibitors of this pathway may prove useful as molecular targeted therapies for SFVMs.

Introduction

Vascular anomalies comprise both malformations and tumors.[1] Vascular malformations have conventionally been classified as slow flow or high flow according to the affected blood flow characteristics.[1] Slow-flow vascular malformations (SFVMs) include venous and lymphatic malformations as well as combined malformations, such as Klippel–Trenaunay syndrome (KTS).[2] SFVMs often cause deformity, pain, chronic anemia, coagulation abnormalities, and functional impairment.[2] Conventional treatments, such as surgery and/or sclerotherapy, are rarely curative, underscoring the urgent need for new therapeutic modalities.[3]

A number of genetic changes have been identified in patients with vascular anomalies, the majority of which occur within two of the major intracellular signaling pathways; namely, the RAS/mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway.[4–6] Recent studies have shown that the PI3K/AKT/mTOR pathway is typically mutated in patients with SFVMs, raising the possibility that targeted therapy could be a useful treatment strategy.[4–6] Indeed, sirolimus, also known as rapamycin, is an allosteric inhibitor of mTOR[7] that has been tested in a monocentric prospective phase II clinical trial for patients with SFVMs that were refractory to standard treatments.[3] Sirolimus was found to be highly effective, resulting in a partial response in all patients, reducing symptoms and increasing quality of life.[3] Genetic analysis has thus advanced our understanding of the mechanisms involved in SFVM pathogenesis and may also suggest new targets for molecular targeted therapies.

In this study, we performed targeted next-generation sequencing of 29 candidate genes associated with vascular anomalies or with the PI3K signaling pathway in affected tissue samples from a cohort of patients with SFVMs.

Results

Patient characteristics and variant identification

Fifty-nine Japanese patients with SFVMs (31 males and 28 females) were included in the study. The patient characteristics are shown in Table 2. Their ages ranged from 0 to 72 years with a median age of 7.5 years. Of the 59 subjects, 21 were diagnosed with venous malformation (VM), 27 with lymphatic malformation (LM), 1 with lymphatic venous malformation (LVM), and 10 with KTS. The diagnosis of VM, LM, LVM, and KTS was based on clinical presentation and radiologic findings and was confirmed by histopathologic examination. Affected tissue samples from all 59 subjects were analyzable. Tissue acquisition methods consisted of surgical excision (n = 46), punch biopsy (n = 12), and needle biopsy (n = 1).

Pathogenic variants were identified in tissues from 38 patients (64.4%) (Table 3). Genes encoding the endothelial receptor tyrosine kinase TIE2 (*TEK*) and the PI3K catalytic subunit α (*PIK3CA*) were the most commonly mutated genes in the study. Eight pathogenic variants in *TEK* and three in *PIK3CA* were identified in 10 samples (16.9%) and 28 samples (47.5%), respectively. We also identified a pathogenic variant *RASA1,* which encodes RAS P21 protein activator 1, in one sample. Single variants of unknown significance were detected in genes encoding MAPK kinase kinase 3 (*MAP3K3* c.1416C>G; p.Ile472Met), Ephrin type-B

receptor 4 (*EPHB4* c.52G>A; p.Glu18Lys), and mTOR (*MTOR* c. 5490_5501del; p.Ala1831_Thr1834del) in one patient with VM and two patients with KTS.

Genetic variants associated with VM

Of the 21 patients with VMs, 14 (66.7%) harbored pathogenic variants (Table 3). The maximum diameter of the lesions ranged from 1.0 to 15.0 cm. Six of the 14 patients showed evidence of multiple lesions. Pathogenic variants in *TEK* were identified in 10 patients, of whom 6 harbored variants in the mutational hotspot Leu914.[5] One of the mutations, c.921C>G; p.Tyr307*, was first identified in patients with vascular anomalies and was shown to result in a premature stop codon, a common feature of pathogenic variants. Two of the 14 patients with VMs possessed multiple pathogenic variants in *TEK* (c.2690A>G; p.Tyr897Cys + c.2752C>T; p.Arg918Cys, and c.2689T>C; p.Tyr897His + c.2753G>T; p.Arg918Leu + c.921C>G; p.Tyr307*).

Five patients with VMs harbored pathogenic variants in *PIK3CA*, all of which were hotspot variants: one c.1624G>A; p.Glu542Lys, three c.1633G>A; p.Glu545Lys, and one c.3140A>G; p.His1047Arg.[24] One patient had a pathogenic variant in both *TEK* (c. 2740C>T; p.Leu914Phe) and *PIK3CA* (c.1633G>A; p.Glu545Lys). Allele frequencies of pathogenic variants in patients with VMs ranged from 3.7% to 13.6%, with 64.3% and 35.7% of individuals having VAFs of <10% and <5%, respectively.

Genetic variants associated with LM

Of the 27 patients with LMs, 15 (55.6%) harbored pathogenic variants (Table 3). The maximum diameter of the lesions ranged from 1.0 to 15.0 cm. One patient showed evidence of multiple lesion. Pathogenic variants in *PIK3CA* were identified in 14 patients with LMs, all of which were hotspot mutations: five c.1624G>A; p.Glu542Lys, four c.1633G>A; p.Glu545Lys, and five c.3140A>G; p.His1047Arg.[6] One patient had a *RASA1* c.1772G>A; p.Arg591His variant. To our knowledge, this variant has not previously been reported in patients with vascular anomalies. *RASA1* c.1772G>A; p.Arg591His is listed in COSMIC[25] as a mutation in angiosarcoma and stomach cancer, although it is indicated to be of uncertain significance in ClinVar[13] and InterVar.[15] *In silico* analyses predicted this variant to be tolerated by SIFT,[21] probably damaging by PolyPhen-2,[22] and disease causing by MutationTaster,[23] and was considered as potentially pathogenic. Allele frequencies of pathogenic variants in patients with LMs ranged from 3.5% to 10.3%, with 93.3% and 60% of individuals having VAFs of <10% and 5%, respectively.

Genetic variants associated with LVM

Only one patient with LVM was included in our study. He harbored a *PIK3CA* hotspot variant (c.1624G>A; p.Glu542Lys) in LVM tissue with a VAF of 17.7% (Table 3). The maximum diameter of the lesion was 23.0 cm.

Genetic variants associated with KTS

Eight of the 10 patients with KTS harbored pathogenic variants in *PIK3CA* (Table 3). All of these were hotspot variants and consisted of two c.1624G>A; p.Glu542Lys, five c.1633G>A; p.Glu545Lys, and one c.3140A>G; p.His1047Arg. Allele frequencies of pathogenic variants in patients with KTS ranged from 6.0% to 17.7%, with 75% and 0% of individuals having VAFs of <10% and <5%, respectively.

Discussion

We report the results of next-generation sequencing of 29 candidate genes in affected tissues from 59 patients with SFVMs. Consistent with the results of previous studies,[4–6] we identified three somatic *PIK3CA* variants (c.1624G>A; p.Glu542Lys, c.1633G>A; p.Glu545Lys, and c.3140A>G; p.His1047Arg) in patients with VM, LM, LVM, and KTS, and eight somatic *TEK* variants (c.2690A>G; p.Tyr897Cys, c.2689T>C; p.Tyr897His, c.2740C>T; p.Leu914Phe, c.2743C>T; p.Arg915C, c.2752C>T; p.Arg918Cys, c.2753G>T; p.Arg918Leu, and c.3295C>T; p.Arg1099*) in patients with VM.

VMs are painful and deforming lesions caused by dilated vascular channels.[27] Somatic activating variants in *TEK* have previously been identified in about 60% of VMs.[27] More recently, somatic activating variants in *PIK3CA* have been identified in about 25% of VM cases.[24] In patients with wild-type *TEK* and *PIK3CA* genes, the VMs are likely caused by infrequent variants in

other genes connected to the PI3K/AKT/mTOR and RAS/MAPK pathways, as suggested by Castel *et al*.[8] *TEK* and *PIK3CA* variants drive constitutive activation of the PI3K/AKT/mTOR pathway, resulting in increased proliferation and survival of endothelial cells, which could account for the increased accumulation of endothelial cells observed in VMs.[8] In two patients with VMs, we detected multiple pathogenic variants in *TEK*. The ratio of single/multiple *TEK* variants in VMs in our study (80%/20%) was similar to that (85.7%/14.3%) reported by Limaye *et al*.[28] These data indicated that the somatic "second hit" might play a determining role in a few of VMs.[28] *TEK* and *PIK3CA* variants are typically mutually exclusive but both occur in some patients with VMs,[8,29,30] as was the case for one patient with hotspot variants of both *TEK* and *PIK3CA* in the present study. It is likely that this can be explained by the presence of two related events in the same cells, because the VAFs of both variants were similar in our case. Another possibility is that variants in *TEK* and *PIK3CA* do not co-exist in the same cell. Further studies are needed to clarify the roles of simultaneous pathogenic variants in *TEK* and *PIK3CA* in the development of VM.

The *TEK* nonsense mutation c.921C>G: p.Tyr307*, identified in one VM patient in the present study, has previously been associated with primary congenital glaucoma.[31] This variant is located in the ectodomain of TEK and is reportedly loss of function.[31] This differs strikingly from *TEK* variants linked to hereditary and sporadic VMs,[27] which are located solely in the intracellular domain and result in enhanced kinase activity.[32] Interestingly, Limaye *et al.* identified a somatic second hit in *TEK*, a loss-of-function deletion, in a VM lesion from a patient carrying the *TEK* R849W variant in the germline.[28] This would suggest that loss-of-function variants in *TEK* might potentiate the development of VMs[33] and thus also implicates the nonsense variant detected in our study might be associated with development of VMs. Further investigation will be needed to clarify the pathogenicity of this variant.

LMs are characterized by the presence of abnormal lymphatic vessels with progressive cystic dilation.[34] Upregulation of the PI3K/AKT/mTOR pathway may be a causal factor in the development of the abnormal lymphatic vessels.[35] Previous studies performed on LM specimens have identified somatic activating variants in the *PIK3CA* gene.[35] For example, Blesinger *et al.* showed that activating *PIK3CA* variants in patients with LM were specifically localized in lymphatic endothelial cells.[36] Using deep targeted sequencing methods with a cohort of 64 patients, Zenner *et al.* reported *PIK3CA* variants in LM tissues from 68.8% of the patients with a VAF of <5%.[37] In our study, 60% (9/15) of the individuals with VMs had a maximum VAF of <5%. These data highlight the need for optimization of DNA sequencing methods to enable detection of very low VAFs in LM tissues.

One of our patients harbored a variant in *RASA1*, which encodes p120-RasGAP protein that inhibits activity of RAS protein.[38] Variants in this gene have been reported to be associated with capillary malformation-arteriovenous malformation and Parkes Weber syndrome, a congenital vascular malformation consisting of capillary malformation, VM, LM, and arteriovenous malformation.[38,39] Most of the *RASA1* mutations responsible for capillary malformation-arteriovenous malformation and Parkes Weber syndrome are loss of function mutations and may lead to activation of RAS and increase downstream signaling via MAPK and PI3K/AKT/mTOR pathways.[38] The *RASA1* missense variant (c.1772G>A; p.Arg591His) identified in our LM patient is potentially pathogenic according to two prediction tools, but further genetic and functional studies are needed to determine whether the variant is indeed pathogenic.

LVMs composed of combined lymphatic and venous elements are present at birth and develop due to errors in venolymphatic development.[26] Two hypotheses have been proposed for the pathogenesis of LVMs. One suggests that the condition results from malformation of lymphatic vascular pathways, while the second considers that it represents a tumor that grows by cellular (mainly endothelial) hyperplasia.[26] A somatic variant of *PIK3CA* was identified in affected tissues from a LVM patient in the present study; however, the cell type harboring the variant is unknown. Further research is needed to elucidate the cellular and molecular pathways driving LVM pathogenesis.

KTS is a syndrome involving capillary and venous malformations as well as limb overgrowth with or without LM.[40] KTS is caused by a mutation in primitive limb-forming cells that are destined to become blood and lymphatic vessels, fat, and bones.[35] In most cases of KTS analyzed to date, the cause is mosaic activating variants of *PIK3CA*.[35] In the present study, we identified pathogenic *PIK3CA* variants in 8 of the 10 patients with KTS.

In recent years, sirolimus has emerged as a new medical treatment option for SFVMs through inhibiting the PI3K/AKT/mTOR signaling pathway.[3] Notably, sirolimus has demonstrated substantial clinical benefit, as reflected by a decrease in the size of most lesions and an improvement in quality of life.[41] However, long-term sirolimus treatment may cause significant side effects due to immunosuppression, and the clinical studies performed thus far suggest that it does not always reduce the volume of existing SFVM lesions.[42] Direct targeting of chronically activated TIE2 and/or PIK3CA kinases using specific inhibitors may provide the best clinical response for patients with SFVMs.[42]

There are several possible reasons why genetic alterations were not detected in 21 of the 59 patients in the present study. For example, the pathogenic alterations could be located in genes other than the 29 investigated here, or they could be located in other gene regions, such as deep intronic regions, not included in our targeted next-generation sequencing approach. Alternatively, the VAFs may have been below the detection limit, the changes may have been epigenetic alterations or large deletions that were undetected using the current sequencing methodology, or sampling errors may have occurred.

In conclusion, pathogenic variants in genes involved in the PI3K signaling pathway were predominant among the 29 genes and 59 samples examined here. Inhibitors of this pathway may therefore have utility as molecular targeted treatments for SFVMs.

Materials And Methods

Patients and tissue samples

Patients diagnosed with SFVMs at six academic hospitals in Japan were enrolled in this study. Sample acquisition and genetic analyses were approved by the institutional review board at each institution, and written informed consent was obtained from all patients or their guardians. Tissue samples were collected during clinically indicated surgical procedures (surgical excision, punch biopsy, and needle biopsy) for therapeutic or diagnostic purposes. Areas of necrosis or hemorrhage on gross inspection were removed from the fresh specimens. Tissues were rapidly frozen in liquid nitrogen until analyzed.

DNA extraction and next-generation sequencing

Genomic DNA was extracted from frozen tissues using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) or NucleoSpin® Tissue kit (Macherey-Nagel, Duren, Germany) according to the manufacturers' protocols. For next-generation sequencing, primer pairs were designed to amplify the exonic regions of 29 candidate genes associated with vascular anomalies or the PI3K signaling pathway (listed in Table 1) using the Ion AmpliSeq Designer (v7.24, Thermo Fisher Scientific, Waltham, MA, USA).[5,6,8,9,10] A total of 1297 primer pairs with 125–275 bp amplicons were synthesized to cover 99.24% of the exonic regions of the 29 genes. Libraries for sequencing were prepared from 20 ng of input purified genomic DNA using the Ion AmpliSeq™ Library Kit Plus (Thermo Fisher Scientific) and the indicated primer pairs, according to the manufacturer's protocol. The libraries were subsequently sequenced on an Ion Proton™ Sequencer (Thermo Fisher Scientific) using the Ion PI™ Chip v3 (Thermo Fisher Scientific) with 15–16 samples per chip.

Sequencing data analysis and variant annotation

The sequencing data were processed using a standard procedure with Ion Torrent Suite Software, and mutations were called using the Torrent Variant Caller plug-in. Called mutations were annotated with SnpEff,[11] SnpSift,[12] and ClinVar.[13] To remove error mutations from called sites and to identify somatic variants, mutations were selected to satisfy the following criteria: sequencing depths of 1000 or more, variant allele frequencies (VAFs) of ≥1% and <45%, and base qualities of ≥50. Mutations resulting in peptide alterations were annotated with dbNSFP[14] to identify single nucleotide variants or with ClinVar[13] to find insertion/deletions and were selected as the functional candidates. Candidate mutations were manually reviewed using Integrative Genomics Viewer.[15] Variants were excluded if they were reported as benign or likely benign in ClinVar[13] or InterVar[16] and their CADD Phred scores[17] were <20, as previously reported.[18] Then, as previously described,[19] variants were denoted as pathogenic if one or more of the following criteria was satisfied: (i) the nucleotide sequence change (or affected amino acid residue) had previously been documented to be pathogenic, (ii) the change resulted in a shift of the transcript open reading frame, (iii) the change introduced a premature stop codon, (iv) the change altered the canonical splice-site sequence, and (v) the

change was a start- or stop-loss mutation. In addition, new missense variants having a minor allele frequency of <0.01 in the 1000 Genomes Project database[20] and having deleterious effects predicted by at least two of three *in silico* pathogenicity prediction tools (SIFT,[21] PolyPhen-2,[22] and MutationTaster[23]) were considered potentially pathogenic variants.

Declarations

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Authors' contributions

A.N., A.F., S.Y., S.S., A.K., F.S., N.A., K.K., K.S., and M.O. conceived and designed the study. K.S. performed the next-generation sequencing and A.N., K.K., K.S., H.O., Y.A., and M.O. interpreted the data. A.N. and M.O. wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

K.K. is an employee of ARTham Therapeutics, Inc. K.S. is an employee of Axcelead Drug Discovery Partners, Inc. The other authors have no conflicts of interest or funding to disclose.

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Availability of data and materials

The datasets and analysis performed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the ethics committee or institutional review board of each institution (Central ethics committee of Gifu University, Tohoku University Research Ethics Committee, Ethics committee of the National Center for Child Health and Development, Shinshu University Ethics Committee, Oita University Faculty of Medicine Ethics Committee, and Keio University School of Medicine Ethics Committee).

Consent to participate

The patients or their legal guardians gave their informed consent to be included.

Consent for publication

The patients or their legal guardians provided written informed consent for the publication of this study.

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Tables

Table 1 Genes analyzed in this study

Gene	Related disorders/diseases
AGGF1	KTS
AKT1	Proteus syndrome
AKT2	VM
AKT3	VM
BRAF	AVM
EPHB4	CM-AVM
GNA11	CH, CM with bone and/or soft tissue hyperplasia, diffuse CM with overgrowth
GNAQ	CH, CM “port–wine” stain, nonsyndromic CM, CM of Sturge–Weber syndrome
HRAS	AVM
IDH1	MS, SCH
IDH2	MS, SCH
IGF1R	see *PIK3CA*[a]
KRAS	AVM
MAP2K1	AVM
MAP3K3	Verrucous VM
MTOR	see *PIK3CA*[b]
NF1	see *PIK3CA*[a]
NRAS	Pyogenic granuloma
PIK3CA	Common (cystic) lymphatic malformation, common VM, KTS
PIK3CB	see *PIK3CA*[b]
PIK3R1	see *PIK3CA*[b]
PIK3R2	see *PIK3CA*[b]
PIK3R3	see *PIK3CA*[b]
PTEN	Bannayan–Riley–Ruvalcaba syndrome, hamartoma of soft tissue/angiomatosis of soft tissue
RASA1	CM-AVM, Parkes Weber syndrome
RICTOR	see *PIK3CA*[b]
RPTOR	see *PIK3CA*[b]
STAMBP	Microcephaly-capillary malformation syndrome
TEK	Common VM, familial cutaneomucosal VM, blue rubber bleb nevus syndrome

Abbreviations: AVM, arteriovenous malformation; CH, congenital hemangioma; CM, capillary malformation; KTS, Klippel–Trenaunay syndrome; MS, Maffucci syndrome; SCH, spindle cell hemangioma; VM, venous malformation. [a]Gene located upstream of the PI3K signaling pathway. [b]Gene located within the PI3K signaling pathway.

Table 3 Summary of pathogenic variants detected in this study

Patient	SFVM type	Gender	Age (years)	Site	Maximum diameter (cm)	Number of lesions	Specimen type	Gene	Variant	VAF (%)
VM1	VM	Male	2	Femur	4.5	Single	Excision	*TEK*	c. 2740C>T; p.Leu914Phe	9.4
VM2	VM	Male	12	Neck	1.0	Multiple	Excision	*TEK*	c. 2740C>T; p.Leu914Phe	10.3
VM3	VM	Male	16	Back	10.0	Single	Punch biopsy	*TEK*	c. 2740C>T; p.Leu914Phe	9.1
VM4	VM	Male	54	Lip	4.0	Multiple	Excision	*TEK*	c. 2740C>T; p.Leu914Phe	4.2
VM5	VM	Female	43	Neck	4.0	Multiple	Excision	*TEK*	c.2743C>T; p.Arg915Cys	12.6
VM6	VM	Female	8	Pelvis	5.0	Multiple	Excision	*TEK*	c.3295C>T; p.Arg1099*	3.7
VM7	VM	Female	2	Leg	4.0	Single	Excision	*TEK*	c.2690A>G; p.Tyr897Cys	4.1
									c.2752C>T; p.Arg918Cys	4.3
VM8	VM	Female	12	Leg	1.0	Single	Punch biopsy	*TEK*	c.2740C>T; p.Leu914Phe	13.6
VM9	VM	Male	5	Head	3.0	Single	Excision	*TEK*	c.2689T>C; p.Tyr897His	8.6
									c.2753G>T; p.Arg918Leu	9.0
									c.921C>G; p.Tyr307*	4.0
VM10	VM	Male	50	Arm	5.0	Single	Excision	*TEK*	c. 2740C>T; p.Leu914Phe	3.8
								PIK3CA	c.1633G>A; p.Glu545Lys	4.0
VM11	VM	Female	17	Clavicle	4.0	Single	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	5.3
VM12	VM	Female	20	Femur	4.0	Multiple	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	11.8
VM13	VM	Male	5	Axilla	15.0	Multiple	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	13.0
VM14	VM	Female	11	Femur	12.8	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	9.2
LM1	LM	Female	1	Buttock	15.0	Single	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	10.3
LM2	LM	Male	1	Auricle	3.0	Single	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	7.9
LM3	LM	Male	0	Neck	15.0	Single	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	4.6
LM4	LM	Female	2	Neck	10.0	Single	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	4.6
LM5	LM	Male	8	Wrist	2.0	Multiple	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	3.5

LM6	LM	Female	1	Leg	5.0	Single	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	4.2
LM7	LM	Female	19	Mesentery	5.0	Single	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	6.7
LM8	LM	Male	5	Cheek	8.0	Single	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	3.8
LM9	LM	Male	1	Cheek	10.0	Single	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	4.0
LM10	LM	Male	6	Tongue	1.0	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	8.4
LM11	LM	Female	0	Neck	2.0	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	4.1
LM12	LM	Male	16	Cheek	5.0	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	4.2
LM13	LM	Male	5	Neck	10.0	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	8.8
LM14	LM	Female	1	Finger	2.0	Single	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	6.2
LM15	LM	Female	4	Foot	5.0	Single	Excision	*RASA1*	c.1772G>A; p.Arg591His	3.6
LVM1	LVM	Male	66	Waist	23.0	Single	Needle biopsy	*PIK3CA*	c.1624G>A; p.Glu542Lys	17.7
KTS1	KTS	Female	17	Buttock	-	-	Punch biopsy	*PIK3CA*	c.1624G>A; p.Glu542Lys	6.0
KTS2	KTS	Female	5	Leg	-	-	Excision	*PIK3CA*	c.1624G>A; p.Glu542Lys	8.9
KTS3	KTS	Female	19	Abdomen	-	-	Punch biopsy	*PIK3CA*	c.1633G>A; p.Glu545Lys	7.4
KTS4	KTS	Male	4	Femur	-	-	Punch biopsy	*PIK3CA*	c.1633G>A; p.Glu545Lys	7.8
KTS5	KTS	Male	1	Leg	-	-	Punch biopsy	*PIK3CA*	c.1633G>A; p.Glu545Lys	17.7
KTS6	KTS	Female	1	Leg	-	-	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	7.4
KTS7	KTS	Female	4	Abdomen	-	-	Excision	*PIK3CA*	c.1633G>A; p.Glu545Lys	8.1
KTS8	KTS	Female	1	Leg	-	-	Excision	*PIK3CA*	c.3140A>G; p.His1047Arg	10.5

Abbreviations: KTS, Klippel–Trenaunay syndrome; LM, lymphatic malformation; LVM, lymphatic venous malformation; SFVM slow-flow vascular malformation; VAF, variant allele frequency; VM, venous malformation.

Due to technical limitations, Table 2 is only available as a download in the Supplemental Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2ClinicopathologicalfeaturesofpatientswithSFVMsubtypes.docx