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# Results and Clinical Impact of a Targeted Next Generation Sequencing Panel for Somatic Overgrowth and Vascular Anomalies

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## Introduction

Vascular anomalies (VA) encompass a variety of neoplastic and developmental defects of the vasculature. Our understanding of the genetic mutations that drive VAs has advanced significantly, allowing for substantial changes in the classification, management and treatment. Molecular characterization is now being incorporated into the diagnostic workup. Here, we share our experience with a next-generation sequencing (NGS) based somatic overgrowth and vascular anomalies (SOVA) panel.

## Methods

A retrospective review was performed of cases submitted for our SOVA panel between 9/1/2020 and 4/30/2021. Testing was performed on lesional tissue from a VA or region of overgrowth in the setting of a VA. Cases were reviewed for specimen characteristics, panel results, and clinical features and impact. The custom SOVA panel covers coding exons of 63 clinically relevant genes with a limit of detection of 5% variant allele frequency (VAF). Testing is performed on a hybrid capture-based NGS platform and analyzed using an in-house bioinformatics pipeline designed for detection of somatic and germline variants. Variants are classified using the published ACMG/AMP guidelines.

## Results

- Clinically significant mutations were found in 19 patients (70%)
- The most common was mosaic gain–of–function mutations in *PIK3CA* (n=7)
- Suspected mosaic mutations were also found in *TEK*, *MAP2K1*, *BRAF*, *KRAS*, *HRAS*, *GNAQ*, and *GNA11*
- Suspected germline mutations were detected in *GLMN* and *PIK3R2*
- 3/27 only a variant of unknown significance was identified at a VAF suggestive of germline
- Tissue source was fresh/frozen in 19 cases and from a paraffin block in 8 cases
- No variants were identified in 5 fresh/frozen tissue specimens, which may not have included sufficient lesion for detection of a mutation

**Case with Clinically Significant Variants Identified**

| Case | Specimen tested/Clinical picture | Gene | Variant(s) detected | VAF |
|---|---|---|---|---|
| 1 | Infantile hemangioma of forehead | *PIK3CA* | c.3141T>G; p.H1047Q | 8.3% |
| 2 | Klippel-Trenauney syndrome | *PIK3CA* | c.1624G>A; p.E542K | 7.5% |
| 3 | Clinically recurrent, arteriovenous malformation of the oral cavity | *MAP2K1* | c.167A>C; p.Q56P | 7.7% |
| 4 | Pyogenic granuloma of right middle finger | *BRAF* | c.1799T>A; p.V600E | 8.1% |
| 5 | Epidermal nevus of lip/oral cavity | *KRAS* | c.35G>A; p.G12D | 25.0% |
| 6 | Multifocal lymphatic malformation of left buttock and thigh | *PIK3R2* VUS (interpreted as disease-causing in patient) | c.1243G>A; p.A415T | 47.7% |
| 7 | Multifocal glomovenous malformation of right thigh | *GLMN* | c.157_161del;p.K53* | 46.0% |
| 8 | Probable CLOVES syndrome | *PIK3CA* | c.3129G>A; p.M1043I | 18.4% |
| 9 | Venous malformation of right face/preauricular | *GNA11* | c.626A>T; p.Q209L | 9.5% |
| 10 | Venous malformation of right forearm | 2 *TEK* mutations | c.2690A>G; p.Y897C | 14.0% |
| | | | c.3288del; p.E1097Rfs*7 | 15.0% |
| 11 | Venous malformation of right arm | *TEK* | c.2740C>T; p.L914F | 5.6% |
| 12 | Atypical vascular and lipoid proliferation of the thigh | *HRAS* in-frame | c.217_218ins27; p.M72_R73insPSAMRDQYM | 7.4% |
| 13 | Sturge Weber Syndrome | *GNAQ* | c.548G>A; p.R183Q | 4.8% |
| 14 | Fibroadipose vascular anomaly of left medial thigh | *PIK3CA* | c.1624G>A; p.E542K | 8.9% |
| 15 | Complex vascular lesion with predominantly lymphatics as well as atypical arterial, venous, and capillary structures | *PIK3CA* | c.1633G>A; p.E545K | 8.7% |
| 16 | Multifocal glomovenous malformation of left arm | *GLMN* | c.157_161del; p.K53* | 66.4% |
| 17 | Megalencephaly Capillary Malformation (MCAP or M-CM) syndrome | *PIK3CA* | c.2908G>A; p.E970K | 11.3% |
| 18 | CLOVES syndrome | *PIK3CA* | c.1357G>A; p.E453K | 8.0% |
| 19 | Venocapillary malformation of left leg | 2 *TEK* mutations | c.2690A>G; p.Y897C | 13.3% |
| | | | c.3292del; p.E1098Sfs*6 | 10.6% |

## Discussion

Molecular characterization has been helpful to more accurately classify VAs. The majority of cases yielded clinically significant mutations, most with a relatively low VAF (range 5-25% for suspected mosaic mutations), highlighting the importance of a somatic-focused platform. Clinical impact included confirmation of clinically suspected syndromes, including *GNAQ* in Sturge-Weber, *PIK3CA* in CLOVES and megalencephaly capillary malformation, and *GLMN* in syndromic glomuvenous malformations. With the ongoing investigation of targeted therapies, clinically significant variants in patients with VAs can guide treatment. One patient with a *MAP2K1* mutation in an arteriovenous malformation was initiated on MEK-inhibitor therapy based on the mutation and additional patients are being considered for PIK3CA-inhibitor clinical trials.

## References

1. Borst AJ, Nakano TA, Blei F, Adams DM, Duis J. A Primer on a Comprehensive Genetic Approach to Vascular Anomalies. Front Pediatr. 2020 Oct 19;8:579591. doi: 10.3389/fped.2020.579591. PMID: 33194911; PMCID: PMC7604490.
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