Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

**Patient: Patient, Example**

| | |
|---|---|
| **DOB** | 7/24/2012 |
| **Gender:** | Female |
| **Patient Identifiers:** | 01234567890ABCD, 012345 |
| **Visit Number (FIN):** | 01234567890ABCD |
| **Collection Date:** | 00/00/0000 00:00 |

## Vascular Malformations Panel, Sequencing and Deletion/Duplication

ARUP test code 2007384

| | |
|---|---|
| Vascular Malformations Panel Specimen | Whole Blood |
| Vascular Malformations Panel Interp | See Note |

RESULT
Two variants of uncertain significance were detected, one each in the ACVRL1 and SMAD9 genes.

VARIANT OF UNCERTAIN SIGNIFICANCE
Gene: ACVRL1 (NM_000020.3)
Nucleic Acid Change: c.1249A>T; Heterozygous
Amino Acid Alteration: p.Ile417Phe
Inheritance: Autosomal dominant

VARIANT OF UNCERTAIN SIGNIFICANCE
Gene: SMAD9 (NM_001127217.3)
Nucleic Acid Change: c.1161C>A; Heterozygous
Amino Acid Alteration: p.Asn387Lys
Inheritance: Autosomal dominant

INTERPRETATION
One variant of uncertain clinical significance, c.1249A>T; p.Ile417Phe, was detected in the ACVRL1 gene by massively parallel sequencing. Pathogenic variants in ACVRL1 are associated with autosomal dominant hereditary hemorrhagic telangiectasia type 2 (MIM: 600376). However, it is uncertain whether this variant is disease-associated or benign.

One variant of uncertain clinical significance, c.1161C>A; p.Asn387Lys, was detected in the SMAD9 gene by massively parallel sequencing. Pathogenic variants in SMAD9 are associated with autosomal dominant primary pulmonary hypertension 2 (MIM: 615342). However, it is uncertain whether this variant is disease-associated or benign.

Please refer to the background information included in this report for a list of the genes analyzed, methodology, and limitations of this test.

Evidence for variant classification:
The ACVRL1 c.1249A>T; p.Ile417Phe variant (rs141653630) is reported in the literature in two individuals affected with telangiectasias; however, one of these individuals also had a pathogenic variant in ENG that explained the phenotype (Alaa El Din 2015, McDonald 2011). This variant is also reported in ClinVar (Variation ID: 599332) and is found in the general population with an allele frequency of 0.018% (50/282,868 alleles) in the Genome Aggregation Database. Functional analyses suggest that this variant does not alter the expression or

function of the ACVRL1 protein, but it is not known if these assays correlate with clinical outcome (Alaa El Din 2015). Computational analyses predict that this variant is deleterious (REVEL: 0.773). Due to limited information, the clinical significance of this variant is uncertain at this time.

The SMAD9 c.1161C>A; p.Asn387Lys variant (rs562953157), to our knowledge, is not reported in the medical literature in individuals with a SMAD9 related disorder, but it is reported in ClinVar (Variation ID: 1297487). This variant is also found in the non-Finnish European population with an allele frequency of 0.021% (27/129,098 alleles) in the Genome Aggregation Database. Computational analyses predict that this variant is deleterious (REVEL: 0.822). Although the frequency of this variant in the non-Finnish European population suggests that it is unlikely to cause primary pulmonary hypertension, the significance of this variant is uncertain at this time.

RECOMMENDATIONS
Medical screening and management should rely on clinical findings and family history. Genetic consultation is recommended. Surveillance of the literature for new information concerning the uncertain variants is recommended.

COMMENTS
Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations:
NONE

REFERENCES
Alaa El Din F et al. Functional and splicing defect analysis of 23 ACVRL1 mutations in a cohort of patients affected by Hereditary Hemorrhagic Telangiectasia. PLoS One. 2015 Jul 15;10(7):e0132111. PMID: 26176610.

McDonald J et al. Molecular diagnosis in hereditary hemorrhagic telangiectasia: findings in a series tested simultaneously by sequencing and deletion/duplication analysis. Clin Genet. 2011 Apr;79(4):335-44. PMID: 21158752.

This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Vascular Malformations Panel, Sequencing and Deletion/Duplication
CHARACTERISTICS: Pathogenic variants in vascular malformation genes lead to defects of blood vessels, causing fast-flow or slow-flow lesions, shunting, swelling, or skin findings. For some disorders, this may lead to potentially life-threatening hemorrhage, stroke, or heart failure.

EPIDEMIOLOGY: The prevalence of hereditary hemorrhagic telangiectasia (HHT) is estimated to be 1 in 5,000 to 1 in 10,000; familial cerebral cavernous malformation (CCM) is 1 in 2,000 to 1 in 10,000; RASA1-CM-AVM is approximately 1 in 20,000; EPHB4-CM-AVM is approximately 1 in 12,000; PTEN hamartoma tumor syndrome is 1 in 200,000; and AKT1-related proteus syndrome is less than 1 in 1,000,000.

INHERITANCE: Autosomal dominant and/or autosomal recessive, depending on the causative gene

PENETRANCE: All conditions exhibit age-related penetrance.

GENES TESTED: ACVRL1, AKT1, BMPR2, CCBE1, CCM2*, EIF2AK4, ELMO2, ENG*, EPHB4, FAT4, FLT4*, FOXC2, GATA2, GDF2, GJC2*, GLMN*, KCNK3, KRIT1, PDCD10, PIEZO1*, PTEN*, RASA1, SMAD4, SMAD9, SOX18*, STAMBP*, TEK, VEGFC

* - One or more exons are not covered by sequencing and/or deletion/duplication analysis for the indicated gene; see

limitations section below.

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the targeted genes (including the 5' UTR of ENG, a region of ACVRL1 intron 9 encompassing the CT-rich variant hotspot region, and selected PTEN promoter variants), followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the indicated genes. Large deletions/duplications confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions of 2 exons or larger are detected with sensitivity greater than 97 percent; single exon deletions are detected with 62 percent sensitivity. Duplications of 3 exons or larger are detected at greater than 83 percent sensitivity. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude a heritable vascular malformation disorder. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes, unless otherwise noted in the methodology section above. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified. Precise breakpoints for large deletions or duplications are not determined in this assay and single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement. The actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s) reported. This test is not intended to detect duplications of 2 or fewer exons in size, though these may be identified. Single exon deletions are reported but called at a lower sensitivity. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) variants, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:
CCM2(NM_001363458) exon(s) 7
CCM2(NM_001363459) exon(s) 6
FLT4(NM_001354989) exon(s) 30
GJC2(NM_020435) partial exon(s) 2(Chr1:228346380-228346419)
PTEN(NM_000314) exon(s) 9
PTEN(NM_001304717) exon(s) 10
PTEN(NM_001304718) exon(s) 9
SOX18(NM_018419) partial exon(s) 1(Chr20:62680707-62680791)
STAMBP(NM_001353969) exon(s) 10
STAMBP(NM_001353970) exon(s) 11
STAMBP(NM_001353976) exon(s) 10

Single exon deletions/duplications will not be called for the following exons:
CCM2(NM_001363458) 7; CCM2(NM_001363459) 6; ENG(NM_001114753) 1; ENG(NM_000118) 1; FLT4(NM_001354989) 30; GLMN(NM_053274) 16;

GLMN(NM_001319683) 15; PIEZO1(NM_001142864) 1,25,47; PTEN(NM_000314) 9; PTEN(NM_001304717) 1,10; PTEN(NM_001304718) 9; STAMBP(NM_001353969) 10; STAMBP(NM_001353970) 11; STAMBP(NM_001353976) 10

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

| Procedure | Accession | Collected | Received | Verified/Reported |
|---|---|---|---|---|
| Vascular Malformations Panel Specimen | 23-031-151539 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |
| Vascular Malformations Panel Interp | 23-031-151539 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |

END OF CHART

**H=High, L=Low, *=Abnormal, C=Critical**