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

Physician: Doctor, Example

Patient: Patient, Example

DOB	1/17/2002
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	01/01/2017 12:34

Vascular Malformations Panel, Sequencing and Deletion/Duplication

ARUP test code 2007384

Vascular Malformations Panel Specimen	Whole Blood

Vascular Malformations Panel Interp

Positive

INDICATION FOR TESTING
Possible hereditary hemorrhagic telangiectasia (HHT).

RESULT
One pathogenic variant was detected in the SMAD4 gene. One variant of uncertain significance was detected in the PTEN gene.

PATHOGENIC VARIANT
Gene: SMAD4 (NM_005359.5)
Nucleic Acid Change: c.1081C>T; Heterozygous
Amino Acid Alteration: p.Arg361Cys
Inheritance: Autosomal dominant

VARIANT OF UNCERTAIN SIGNIFICANCE
Gene: PTEN (NM_000314.6)
Nucleic Acid Change: c.538T>C; Heterozygous
Amino Acid Alteration: p.Tyr180His
Inheritance: Autosomal dominant

INTERPRETATION
One copy of a pathogenic variant, c.1081C>T; p.Arg361Cys, was detected in the SMAD4 gene by massively parallel sequencing and confirmed by Sanger sequencing. Pathogenic variants in SMAD4 are inherited in an autosomal dominant manner, and are associated with juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (MIM: 175050). This molecular result, consistent with being affected, should be correlated with this individual's clinical presentation.

One copy of a variant of uncertain clinical significance, c.538T>C; p.Tyr180His, was detected in the PTEN gene by massively parallel sequencing. Pathogenic variants in the PTEN gene are associated with autosomal dominant PTEN Hamartoma Tumor Syndrome (PHTS). Based on currently available information, it is uncertain whether this variant is disease-associated or benign.

No additional pathogenic variants were identified in the targeted genes by massively parallel sequencing or deletion/duplication analysis. Please refer to the background information included in this report for a list of the genes analyzed and limitations of this test.

Evidence for variant classification:
The SMAD4 c.1081C>T; p.Arg361Cys variant (rs80338963) is

reported in the literature in multiple individuals affected with hereditary hemorrhagic telangiectasia (HHT) or juvenile polyposis syndrome (JPS) (Aretz 2007, Gallione 2006, Gallione 2010, Houlston 1998, Woodford-Richens 2001). This variant is absent from general population databases (Exome Variant Server, Genome Aggregation Database), indicating it is not a common polymorphism. The arginine at amino acid 361 is highly conserved, and functional analyses suggest that this variant exhibits deficient homo-oligomerization and deficient binding to other partner proteins (Shi 1997), which may result in instability and degradation (Woodford-Richens 2001). Additionally, other amino acid substitutions at this codon (Gly, His, Leu, Ser) have been reported in individuals with HHT or JPS and are considered disease-causing (Aretz 2007, Gallione 2010, Howe 2004). Based on available information, the p.Arg361Cys variant is considered to be pathogenic.

The PTEN c.538T>C; p.Tyr180His variant (rs746280047), to our knowledge, is not reported in the medical literature but is reported in ClinVar (Variation ID: 481129). This variant is found on a single chromosome in the Genome Aggregation Database (1/251234 alleles), indicating it is not a common polymorphism. The tyrosine at codon 180 is moderately conserved, and computational analyses (SIFT, PolyPhen-2) predict that this variant is tolerated. However, due to limited information, the clinical significance of the p.Tyr180His variant is uncertain at this time.

RECOMMENDATIONS
Genetic consultation is indicated, including a discussion of medical screening and management. At risk family members should be offered testing for the identified pathogenic SMAD4 variant (Familial Mutation, Targeted Sequencing, ARUP test code 2001961). Surveillance of the literature for new information concerning the uncertain PTEN variant is recommended.

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. Likely benign and benign variants are not included in this report.

REFERENCES
Aretz S et al. High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome. J Med Genet. 2007 Nov;44(11):702-9.

Gallione C et al. Overlapping spectra of SMAD4 mutations in juvenile polyposis (JP) and JP-HHT syndrome. Am J Med Genet A. 2010 Feb;152A(2):333-9.

Gallione CJ et al. SMAD4 mutations found in unselected HHT patients. J Med Genet. 2006 Oct;43(10):793-7.

Houlston et al. Mutations in DPC4 (SMAD4) cause juvenile polyposis syndrome, but only account for a minority of cases. Hum Mol Genet. 1998 Nov;7(12):1907-12.

Howe JR et al. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. J Med Genet. 2004 Jul;41(7):484-91.

Shi Y et al. A structural basis for mutational inactivation of the tumour suppressor Smad4. Nature. 1997 Jul 3;388(6637):87-93.

Woodford-Richens KL et al. Comprehensive analysis of SMAD4 mutations and protein expression in juvenile polyposis: evidence for a distinct genetic pathway and polyp morphology in SMAD4 mutation carriers. Am J Pathol. 2001 Oct;159(4):1293-300.

This result has been reviewed and approved by [redacted]

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

[redacted]

BACKGROUND INFORMATION: Vascular Malformations Panel, Sequencing and Deletion/Duplication

CHARACTERISTICS: Defects of either blood or lymphatic vessels. This panel focuses on disorders associated with structural defects of blood vessels.

EPIDEMIOLOGY: The prevalence of hereditary hemorrhagic telangiectasia (HHT) is 1 in 10,000; familial cerebral cavernous malformation (CCM) is 1 in 2,000 to 10,000; RASA1-related disorders (CM-AVM, Parkes Weber) is approximately 1 in 100,000; Pulmonary Arterial Hypertension (PAH) is 1-2/100,000; PTEN-related Proteus syndrome (PS)/Proteus-like syndrome (PLS) is estimated at less than 1 in 1,000,000; and Juvenile polyposis/hereditary hemorrhagic telangiectasia (JP/HHT) syndrome is approximately 1 in 100,000.

INHERITANCE: Autosomal dominant.

PENETRANCE: All exhibit age-related penetrance. 90 percent or greater for HHT, JP/HHT, RASA1-related disorders, VMCM and GVM. 50-75 percent for CCM. 1-20 percent for PAH, depending on gene. Unknown for PTEN-related PS/PLS.

GENES TESTED: ACVRL1, AKT1**, BMPR2, CAV1, CCBE1, CCM2, EIF2AK4**, ELMO2**, ENG, EPHB4**, FAT4**, FLT4, FOXC2, GATA2**, GDF2, GJC2, GLMN, KCNK3, KRIT1, PDCD10, PIEZO1**, PIK3CA***, PTEN, RASA1, SMAD4, SMAD9**, SOX18***, STAMBP**, TEK, VEGFC**

** - Deletion/duplication detection is not available for this gene.
*** - One or more exons are not covered by sequencing, and deletion/duplication detection is not available for this gene; see limitations section below.

METHODOLOGY: Targeted capture of all coding exons and exon-intron junctions of the targeted genes, including the PTEN promoter region, followed by massively parallel sequencing. The 5' untranslated region of ENG and a region of ACVRL1 intron 9 encompassing the CT-rich variant hotspot region were sequenced. Sanger sequencing was performed as necessary to fill in regions of low coverage and confirm reported variants. A custom tiled comparative genomic hybridization array (aCGH) was used to detect large deletions or duplications in the indicated subset of genes. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY: The analytical sensitivity of this test is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions from 1-10 base pairs in size. Variants greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced.

LIMITATIONS: A negative result does not exclude a heritable form of a vascular malformation disorder. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. Regulatory region variants and deep intronic variants will not be identified and breakpoints of large deletions/duplications will not be determined. Single exon deletions/duplications or deletions/duplications less than 1kb may not be detected. Deletions/duplications/insertions of any size may not be detected by massive parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay may not detect low-level somatic variants associated with disease. Interpretation of this test

result may be impacted if this patient has had an allogeneic stem cell transplantation. Non-coding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:
PIK3CA(NM_006218) exon(s) 10,11,12,13,14
SOX18(NM_018419) exon(s) 1

Single exon deletions/duplications will not be called for the following exons:
FLT4(NM_002020) 30;FLT4(NM_182925) 20,22;GLMN(NM_053274) 11;PTEN(NM_000314) 8,9;PTEN(NM_001304717) 1;TEK(NM_000459) 1

Test developed and characteristics determined by ARUP Laboratories. See Compliance Statement C: aruplab.com/CS

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Vascular Malformations Panel Specimen	19-275-403282	10/2/2019 1:02:00 PM	10/4/2019 2:05:00 PM	11/6/2019 8:03:00 AM
Vascular Malformations Panel Interp	19-275-403282	10/2/2019 1:02:00 PM	10/4/2019 2:05:00 PM	11/6/2019 8:03:00 AM

END OF CHART