ARUP LABORATORIES | www.aruplab.com
500 Chipeta Way, Salt Lake City, Utah 84108-1221
phone: (801) 583-2787, toll free: (800) 522-2787
Julio C. Delgado, MD, MS, Director of Laboratories

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

Physician: Doctor, Example

Patient: Patient, Example

DOB	3/9/2019
Gender:	Male
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Rapid Mendelian Genes Sequencing Panel, Trio

ARUP test code 2012849

Rapid Sequencing Specimen	Whole Blood
Rapid Sequencing Interpretation	Positive

Section 79-1 of New York State Civil Rights Law requires informed consent be obtained from patients (or their legal guardians) prior to pursuing genetic testing. These forms must be kept on file by the ordering physician. Consent forms for genetic testing are available at www.aruplab.com. Incidental findings are not reported unless clinically significant but are available upon request.

Test Performed: Rapid Mendelian Genes Sequencing Panel, Trio

Next generation sequencing was performed on 4900+ genes in the patient and this patients unaffected parents. Parental sequencing was performed to aid in variant analysis and result interpretation.

Indication for Testing: [Individual medical history and indication for testing.]

Result: Positive; one pathogenic variant was identified in the RASA1 gene that provides an explanation for this individual's phenotype.

Interpretation: One pathogenic variant was identified in the RASA1 gene that provides an explanation for the patient's symptoms. Pathogenic variants in RASA1 are inherited in an autosomal dominant manner and are associated with capillary malformation-arteriovenous malformations (MIM: 608354), Parkes Weber syndrome (MIM: 608355), and other vascular anomalies (Revencu 2008).

Additionally, one ACMG secondary finding, a likely pathogenic variant in the TNNT2 gene, was also detected. Pathogenic variants in TNNT2 are inherited in an autosomal dominant manner and are associated with dilated cardiomyopathy 1D (MIM: 601494), familial restrictive cardiomyopathy 3 (MIM: 612422), hypertrophic cardiomyopathy 2 (MIM: 115195), and left ventricular noncompaction 6 (MIM: 601494).

PATHOGENIC VARIANT
Gene: RASA1 (NM_002890.2)
Inheritance pattern: Autosomal dominant
Variant: c.1103-2A>C; Heterozygous
Chr5(GRCh37):g.86645029
Frequency: No rs, not listed in gnomAD

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

Paternally Inherited

One paternally inherited pathogenic variant, c.1103-2A>C, was detected in the RAS p21 protein activator 1 (RASA1) gene by massively parallel sequencing and confirmed by Sanger sequencing. Protein encoded by this gene, Ras GTPase-activating protein (RasGAP) converts the Ras small GTP-binding protein from an active GTP-bound to an inactive GDP-bound state during growth factor receptor signal transduction. In addition, RASA1 has also been reported to perform Ras-independent functions in intracellular signal transduction (Lapinki 2018). RASA1 loss-of-function germline variants have been described in RASA1-related disorders with presentations of capillary malformations with or without arteriovenous malformations or arteriovenous fistulas in brain, skin, muscle, bone and spine that may lead to hemorrhage, neurological presentations including epilepsy, seizures, congestive heart failure, respiratory problems, shortness of breath, pulmonary edema, and cutaneous ischemia (Orme 2013, Wooderchak-Donahue 2018). Hypertrophy/overgrowth, chylothorax and lymphedema have also been reported in patients with RASA1 pathogenic variants (Burrows 2013, Macmurdo 2016, Wooderchak-Donahue 2018). Hydrops fetalis has been reported in at least two cases, one of them also with polyhydramnios (Revencu 2013, Overcash 2015). Intrafamilial variability have been documented in several cases (de Wijn 2012, Burrows 2013, Revencu 2013, Overcash 2015, Macmurdo 2016). The somatic, second hit inactivating RASA1 variants identified in endothelial somatic cells (Revencu 2013, Macmurdo 2016, Lapinki 2018) have been postulated as a plausible explanation for the intrafamilial phenotypic variability (Revencu 2013).

The c.1103-2A>C variant has not been reported in the medical literature, gene specific variant databases including ClinVar, nor has it been previously identified by our laboratory. However, a different nucleotide change in the same splice acceptor site, c.1103-2A>G, has been previously identified in our laboratory in a 14 year old individual with capillary malformations, shortness of breath and exercise intolerance (Wooderchak-Donahue 2018). The c.1103-2A>C variant abolishes the splice acceptor site of intron 7 and is predicted to alter splicing (Alamut v.2.11). Based on available information, this variant is considered pathogenic.

No other pathogenic variants were identified that are related to the patient's phenotype.

ACMG Secondary Finding
LIKELY PATHOGENIC VARIANT
Gene: TNNT2 (NM_001001430.2)
Inheritance pattern: Autosomal dominant
Variant: c.1A>G; p.Met1?; Heterozygous
Chr1(GRCh37):g.201342382
Frequency: No rs, not listed in gnomAD

One likely pathogenic variant, c.1A>G, was identified in the Troponin T2, Cardiac (TNNT2) gene by massively parallel sequencing and confirmed by Sanger sequencing. The c.1A>G variant has not been reported in the medical literature, gene specific variant databases including ClinVar, or previously identified by our laboratory. The c.1A>G variant is predicted to cause a start-loss of the TNNT2 gene due to a change of the methionine initiation codon. Even though a large number of pathogenic TNNT2 variants are missense variants, at least ten loss-of-function TNNT2 variants have been reported (Hirtle-Lewis 2013, van Spaendonck-Zwarts 2013, Coppini 2014, Jamuar 2016, Messer 2016, Walsh 2017). Pathogenic variants in TNNT2 are inherited in an autosomal dominant manner and are associated with dilated cardiomyopathy 1D (MIM: 601494), familial restrictive cardiomyopathy 3 (MIM: 612422), hypertrophic cardiomyopathy 2 (MIM: 115195), and left ventricular noncompaction 6 (MIM: 601494).

No other secondary pathogenic variants were detected in the American College of Medical Genetics and Genomics (ACMG) recommended list of genes. This result does not exclude the possibility this individual may have a variant in one of these genes as not all of the ACMG recommended genes are adequately covered by this method, or in another gene not included on this list. Note that single pathogenic variants in recessive genes from this list are not reported.

Recommendations: Genetic consultation is indicated, including a discussion of medical screening and management. At risk family members should be offered targeted testing for the RASA1 and TNNT2 variants (Familial Mutation, Targeted Sequencing, ARUP test code 2001961). Preimplantation or prenatal diagnosis for the identified variants should be offered to the patient and this patients parents for future pregnancies.

References:
Burrows PE et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. Proc Natl Acad Sci U S A. 2013 May 21;110(21):8621-6.
Coppini R et al. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. J Am Coll Cardiol. 2014 Dec 23;64(24):2589-2600.
de Wijn RS et al. Phenotypic variability in a family with capillary malformations caused by a mutation in the RASA1 gene. Eur J Med Genet. 2012 Mar;55(3):191-5.
Durrington HJ et al. A novel RASA1 mutation causing capillary malformation-arteriovenous malformation (CM-AVM) presenting during pregnancy. Am J Med Genet A. 2013 Jul;161A(7):1690-4.
Hirtle-Lewis M et al. The genetics of dilated cardiomyopathy: a prioritized candidate gene study of LMNA, TNNT2, TCAP, and PLN. Clin Cardiol. 2013 Oct;36(10):628-33.
Jamuar SS et al. Incidentalome from Genomic Sequencing: A Barrier to Personalized Medicine? EBioMedicine. 2016 Feb 4;5:211-6.
Lapinski PE et al. Somatic second hit mutation of RASA1 in vascular endothelial cells in capillary malformation-arteriovenous malformation. Eur J Med Genet. 2018 Jan;61(1):11-16.
Macmurdo CF et al. RASA1 somatic mutation and variable expressivity in capillary malformation/arteriovenous malformation (CM/AVM) syndrome. Am J Med Genet A. 2016 Jun;170(6):1450-4.
Messer AE et al. Mutations in troponin T associated with Hypertrophic Cardiomyopathy increase Ca(2+)-sensitivity and suppress the modulation of Ca(2+)-sensitivity by troponin I phosphorylation. Arch Biochem Biophys. 2016 Jul 1;601:113-20.
Orme CM et al. Capillary malformation-arteriovenous malformation syndrome: review of the literature, proposed diagnostic criteria, and recommendations for management. Pediatr Dermatol. 2013 Jul-Aug;30(4):409-15.
Overcash RT et al. Maternal and fetal capillary malformation-arteriovenous malformation (CM-AVM) due to a novel RASA1 mutation presenting with prenatal non-immune hydrops fetalis. Am J Med Genet A.2015 Oct;167A(10):2440-3.
Revencu N et al. Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. Hum Mutat. 2008 Jul;29(7):959-65.
Revencu N et al. RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation. Hum Mutat. 2013 Dec;34(12):1632-41.
van Spaendonck-Zwarts, KY, et al. Genetic analysis in 418 index patients with idiopathic dilated cardiomyopathy: overview of 10 years' experience. Eur J Heart Fail. 2013 Jun;15(6):628-36.
van Velzen HG et al. Outcomes of Contemporary Family Screening in Hypertrophic Cardiomyopathy. Circ Genom Precis Med. 2018 Apr;11(4):e001896.
Walsh R et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet Med. 2017 Feb;19(2):192-203.

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

Wooderchak-Donahue WL et al. Expanding the clinical and molecular findings in RASA1 capillary malformation-arteriovenous malformation. Eur J Hum Genet. 2018.

Please refer to the background information included in this report for limitations of this test.

This result has been reviewed and approved by Rong Mao, M.D.

BACKGROUND INFORMATION: Rapid Mendelian Genes Sequencing Panel, Trio

CHARACTERISTICS: This gene panel was designed for diagnostic testing in critically ill infants suspected of having an underlying genetic diagnosis. Massively parallel sequencing was performed on the DNA coding regions and intron/exon boundaries of genes identified in the Human Genome Mutation Database (HGMD) that are confirmed to have a human disease association. Variants of uncertain significance in recessive genes will be reported when one pathogenic variant has also been identified in the same gene. Parental samples are required for interpretation of results.

CLINICAL SENSITIVITY: Varies based on clinical symptoms, family history, inheritance pattern, previous evaluations and receipt of parental samples.

GENES TESTED: Refer to Additional Technical Information located at http://ltd.aruplab.com/Tests/Pub/2012849.

METHODOLOGY: Targeted capture of all (or selected) coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to confirm reported variants. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: 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 all genetic diagnoses. 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. Deletions/duplications/insertions of any size may not be detected by massively 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. Refer to Additional Technical Information for complete list of genes tested and gene-specific limitations, located at http://ltd.aruplab.com/Tests/Pub/2012849.

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

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

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Rapid Sequencing Specimen	19-070-151131	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Rapid Sequencing Interpretation	19-070-151131	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