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	11/14/2001
Gender:	Male
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Cobalamin/Propionate/Homocysteine Metabolism Related Disorders Panel, Sequencing and Deletion/Duplication

ARUP test code 2011157

Cobalamin/Propionate/Homocysteine Spec: Whole Blood

Cobalamin/Propionate/Homocysteine Int: Positive

INDICATION FOR TESTING
Confirm diagnosis of homocystinuria.

RESULT
Two pathogenic variants were detected in the CBS gene.

PATHOGENIC VARIANT
Gene: CBS (NM_000071.2)
Nucleic Acid Change: c.833T>C; Heterozygous
Amino Acid Alteration: p.Ile278Thr
Inheritance: Autosomal Recessive

PATHOGENIC VARIANT
Gene: CBS (NM_000071.2)
Nucleic Acid Change: c.959T>C; Heterozygous
Amino Acid Alteration: p.Val320Ala
Inheritance: Autosomal Recessive

INTERPRETATION
One copy each of two pathogenic variants, c.833T>C; p.Ile278Thr and c.959T>C; p.Val320Ala, were detected in the CBS gene by massively parallel sequencing and confirmed by Sanger sequencing. Pathogenic CBS variants are inherited in an autosomal recessive manner and are associated with homocystinuria, B6-responsive and nonresponsive types (MIM: 236200). If these variants are present on opposite chromosomes, this molecular result is consistent with a diagnosis of homocystinuria. Parental testing can confirm if these variants are located on the same or opposite chromosomes.

No additional pathogenic variants were identified in the other 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 classifications:
The CBS c.833T>C; p.Ile278Thr variant (rs5742905) is the most frequently reported variant associated with pyridoxine-responsive homocystinuria and has been observed in affected individuals in both the homozygous and compound heterozygous states (Gaustadnes 1999, Refsum 2004, Skovby 2010, Magner 2011, Sorensen 2016). Functional studies demonstrate that the p.Ile278Thr variant has decreased stability and severely

reduced activity relative to wildtype protein (Kozich 2010, Hnizda 2012, Mayfield 2012). The clinical presentation of p.Ile278Thr homozygotes has been described as mild, with many patients having thrombosis as their initial symptom (Skovby 2010). This variant is classified as pathogenic in ClinVar (ID: 120) and is found in the general population with an overall allele frequency of 0.08% (24/30,774 alleles) in the Genome Aggregation Database. The isoleucine at codon 278 is moderately conserved, and computational analyses (SIFT, PolyPhen-2) predict that this variant is deleterious. Based on the available evidence, the p.Ile278Thr variant is considered to be pathogenic.

The CBS c.959T>C; p.Val320Ala variant (rs781567152) is reported in the literature in multiple individuals affected with homocystinuria in both the homozygous state and in individuals with a second pathogenic variant (Kim 1997, Kruger 2003). This variant is reported in ClinVar (Variation ID: 371028), and it is found on only four chromosomes in the Genome Aggregation Database, indicating it is not a common polymorphism. Another variant at this codon (p.Val320Gly) has been reported in an individual with homocystinuria and is considered pathogenic (Lu 2012). The valine at codon 320 is moderately conserved, and computational analyses (SIFT, PolyPhen-2) predict that the p.Val320Ala variant is deleterious. Consistent with these predictions, functional analyses demonstrate that the p.Val320Ala variant exhibits significantly lower enzymatic activity than wildtype protein (Kruger 2003, Singh 2010) and fails to rescue yeast growth on media lacking a source of cysteine (Kim 1997, Mayfield 2012). Based on available information, this variant is considered to be pathogenic.

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 CBS variants (Familial Mutation, Targeted Sequencing, ARUP test code 2001961). Targeted testing for the identified variants in the parents of this individual would determine if the two variants are located on the same or opposite chromosomes.

COMMENTS
Benign variants are not included in this report.

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This result has been reviewed and approved by Weimin Sun, Ph.D.

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

BACKGROUND INFORMATION: Cobalamin/Propionate/Homocysteine Metabolism Related Disorders Panel, Sequencing (25 Genes) and Deletion/ Duplication (24 Genes)

CHARACTERISTICS OF COBALAMIN/PROPIONATE/HOMOCYSTEINE METABOLISM RELATED DISORDERS: Inherited disorders of cobalamin metabolism include defects of absorption, transport and intracellular metabolism. Defects of cobalamin metabolism lead to the accumulation of methylmalonic acid and/or homocysteine in blood and urine. Usually, elevated propionylcarnitine and/or propionyl/acetylcarnitine ratio is detected in plasma and increased methylmalonic acid is detected in blood despite normal or elevated vitamin B12 levels. The clinical features of these disorders are highly variable with onset ranging from the neonatal period to adulthood.

PREVALENCE: The frequency of methylmalonic aciduria from all causes in North America is 1 in 48,000-61,000; isolated methylmalonic acidemia is 1 in 50,000-100,000; combined malonic/methylmalonic aciduria is estimated as 1 in 30,000; methylmalonic aciduria and homocystinuria, cblC type is 1 in 67,000; homocystinuria due to cystathionine beta-synthase deficiency is reported as 1 in 1,800 in Qatar, 1 in 6,400 in Norway, and 1 in 17,800 in Germany; homocystinuria, B6-responsive and nonresponsive types combined are 1 in 58,000-1,000,000; propionic acidemia is 1 in 50,000-100,000 but higher in the Inuit in Greenland with 1 in 1,000-2,000, and in Saudi Arabia with 1 in 5,000. Prevalence is rare for the other disorders included in the panel.

INHERITANCE: X-linked for HCFC1; autosomal dominant or autosomal recessive for MAT1A; and autosomal recessive for all the other genes tested.

PENETRANCE: Reduced for MAT1, MCEE, LMBRD1, and TCN1 genes.

CAUSE: Pathogenic germline mutations in ABCD4, ACSF3, AMN, CBS, CD320, CUBN, GIF, HCFC1, LMBRD1, MAT1A, MCEE, MMAA, MMAB, MMACHC, MMADHC, MTHFR, MTR, MTRR, MUT, PCCA, PCCB, SUCLA2, SUCLG1, TCN1, TCN2 and other genes.

CLINICAL SENSITIVITY: Unknown.

METHODOLOGY: Targeted capture of all coding exons and exon-intron boundaries followed by massively parallel sequencing of ABCD4, ACSF3, AMN, CBS, CD320, CUBN, GIF, HCFC1, LMBRD1, MAT1A, MCEE, MMAA, MMAB, MMACHC, MMADHC, MTHFR, MTR, MTRR, MUT, PCCA, PCCB, SUCLA2, SUCLG1, TCN1 and TCN2. Deletion/duplication analysis of ABCD4, ACSF3, AMN, CBS, CD320, CUBN, GIF, LMBRD1, MAT1A, MCEE, MMAA, MMAB, MMACHC, MMADHC, MTHFR, MTR, MTRR, MUT, PCCA, PCCB, SUCLA2, SUCLG1, TCN1 and TCN2 genes by tiled custom designed comparative genomic hybridization (CGH) array. Sanger sequencing is performed for bases with insufficient coverage and to confirm all reported variants. The Hg 19 Human Genome build is used for data analysis.

LIMITATIONS: Regulatory region mutations, deep intronic mutations, breakpoints of large deletion/duplications, and deletions/duplications in HCFC1gene are not assayed. Small deletions or insertions may not be detected. 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. It is also possible some insertion/deletion variants may not be identified.

See Compliance Statement C: aruplab.com/CS

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Cobalamin/Propionate/Homocysteine Spec	18-229-120260	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Cobalamin/Propionate/Homocysteine Int	18-229-120260	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