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

Physician: Doctor, Example

Patient: Patient, Example

DOB	6/18/1988
Gender:	Male
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Hereditary Gastrointestinal Cancer High-Risk Panel, Sequencing and Deletion/Duplication

ARUP test code 3005697

GIHR Specimen: Whole Blood

GIHR Interp: Positive

RESULT
One pathogenic variant was detected in the MSH6 gene.

PATHOGENIC VARIANT
Gene: MSH6 (NM_000179.3)
Nucleic Acid Change: c.2150_2153del; Heterozygous
Amino Acid Alteration: p.Val717AlafsTer18
Inheritance: Autosomal dominant

INTERPRETATION
One pathogenic variant, c.2150_2153del; p.Val717AlafsTer18, was detected in the MSH6 gene by massively parallel sequencing. This result is consistent with a diagnosis of Lynch syndrome/hereditary non-polyposis colorectal cancer (HNPCC), a hereditary cancer predisposition syndrome. A single pathogenic MSH6 variant increases the risk for colorectal, uterine, and other cancers; lifetime risks for different cancers vary. National Comprehensive Cancer Network (NCCN) guidelines are available for cancer risk management in heterozygous individuals. Other genetic/environmental factors may influence an individual's risk of developing cancer. This individual's offspring have a 50 percent chance of inheriting the pathogenic variant.

In addition, autosomal recessive inheritance of two MSH6 pathogenic variants is associated with constitutional mismatch repair-deficiency (CMMRD), a childhood cancer predisposition syndrome characterized by hematologic, brain, and intestinal tumors (Wimmer 2014, MIM: 276300); thus, this individual is at least a carrier of this disorder.

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 MSH6 c.2150_2153del; p.Val717AlafsTer18 variant (rs267608058), also known as c.2147_2150delCAGT or 2149delTCAG, is reported in the literature in multiple individuals affected with Lynch syndrome and associated cancers (Baglietto 2010, DeRycke 2017, Hirasawa 2017, Kolodner 1999, Nilbert 2009, Pal 2012, Sun 2017, Talseth-Palmer 2010, Walsh 2011). This variant is also reported in ClinVar (Variation ID: 89256). It is only observed on two alleles in the Genome Aggregation Database, indicating it is not a common polymorphism. This variant causes

a frameshift by deleting 4 nucleotides, so it is predicted to result in a truncated protein or mRNA subject to nonsense-mediated decay. Additionally, several downstream truncating variants have been described in individuals with various cancers and are considered pathogenic (Nilbert 2009, Pal 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 MSH6 variant (Familial Targeted Sequencing, ARUP test code 3005867). Counseling for potential reproductive risk associated with CMMRD is recommended (NCCN Guidelines).

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
Baglietto L et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst. 2010 Feb 3;102(3):193-201. PMID: 20028993
DeRycke MS et al. Targeted sequencing of 36 known or putative colorectal cancer susceptibility genes. Mol Genet Genomic Med. 2017 Jul 23;5(5):553-569. PMID: 28944238
Hirasawa A et al. Prevalence of pathogenic germline variants detected by multigene sequencing in unselected Japanese patients with ovarian cancer. Oncotarget. 2017 Nov 28;8(68):112258-112267. PMID: 29348823
Kolodner RD et al. Germ-line msh6 mutations in colorectal cancer families. Cancer Res. 1999 Oct 15;59(20):5068-74. PMID: 10537275
National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Colorectal (2.2023). https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf.
Nilbert M et al. Major contribution from recurrent alterations and MSH6 mutations in the Danish Lynch syndrome population. Fam Cancer. 2009;8(1):75-83. PMID: 18566915
Pal T et al. Frequency of mutations in mismatch repair genes in a population-based study of women with ovarian cancer. Br J Cancer. 2012 Nov 6;107(10):1783-90. PMID: 23047549
Sun J et al. Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients. Clin Cancer Res. 2017 Oct 15;23(20):6113-6119. PMID: 28724667
Talseth-Palmer BA et al. MSH6 and PMS2 mutation positive Australian Lynch syndrome families: novel mutations, cancer risk and age of diagnosis of colorectal cancer. Hered Cancer Clin Pract. 2010 May 21;8(1):5. PMID: 20487569
Walsh T et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A. 2011 Nov 1;108(44):18032-7. PMID: 22006311
Wimmer K et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). Med Genet. 2014 Jun;51(6):355-65. PMID: 24737826.

This result has been reviewed and approved by [redacted].

BACKGROUND INFORMATION: Hereditary Gastrointestinal Cancer High-Risk Panel, Sequencing and Deletion/Duplication
CHARACTERISTICS: Pathogenic germline variants in multiple genes have been implicated in hereditary gastrointestinal (GI) cancer. Hereditary cancer predisposition is often characterized by early age of onset (typically before age 50) and multiple, multifocal,

and/or similar cancers in a single individual or in closely related family member(s). Lynch syndrome (LS), the most common hereditary predisposition to colorectal cancer, is caused by pathogenic germline variants in the MLH1, MSH2, MSH6, PMS2, and EPCAM genes. Pathogenic germline variants in the APC gene are causative for familial adenomatous polyposis (FAP) and other APC-associated polyposis conditions. Biallelic pathogenic germline variants in MUTYH are causative for MUTYH-associated polyposis (MAP).

EPIDEMIOLOGY: Greater than 2-4 percent of colorectal cancers are associated with a hereditary cause. Prevalence of LS in the general population has been estimated at 1 in 279 individuals. The prevalence of FAP has been estimated to be between 1 in 6,850 to 1 in 31,250 live births. The prevalence of MAP is estimated to be between 1 in 20,000 to 1 in 60,000 individuals.

CAUSE: Pathogenic germline variants in genes associated with a high lifetime risk of colorectal cancer.

INHERITANCE: LS and FAP/APC-associated conditions are autosomal dominant. MAP is autosomal recessive.

GENES TESTED: APC*; EPCAM**; MLH1; MSH2; MSH6; MUTYH; PMS2

*One or more exons are not covered by sequencing and/or deletion/duplication analysis for the indicated gene; see limitations section below.
**Deletion/duplication analysis of EPCAM (NM_002354) exon 9 only; sequencing is not available for this gene.

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the targeted genes, 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. Testing of selected exons (and exon/intron boundaries) of PMS2 and MSH2 was performed by bidirectional Sanger sequencing. Deletion/duplication testing of PMS2 was performed by multiplex ligation-dependent probe amplification (MLPA).

ANALYTICAL SENSITIVITY/SPECIFICITY: 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. The analytical sensitivity for MLPA is greater than 99 percent.

LIMITATIONS: A negative result does not exclude a heritable form of cancer. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. 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 two 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:
APC (NM_001354896) exon 12
APC (NM_001354898, NM_001354904) exon 2
APC (NM_001354900) exon 11

Deletions/duplications will not be called for the following exons:
APC (NM_001354896) 12; APC (NM_001354898, NM_001354904) 2; APC (NM_001354900) 11

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. 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
GIHR Specimen	24-026-121314	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
GIHR Interp	24-026-121314	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