

Ordered By Medical Professional: Client: UNKNOWN, UNKNOWN, MD MOCKORG44 (10829)	Contact ID:5075951 Org ID:8141	Patient Legal Name: Unknown, Unknown Accession #: 00-230831 AP2 Order #: 2609139 Birthdate: 02/02/1980 MRN #: N/A Indication: Diagnostic/Family History	Specimen #: Specimen: Blood EDTA (Purple top) Sex assigned at birth: U Collected: 08/21/2024 Received: 08/23/2024 Test Started: 08/23/2024
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CancerNext® +RNAinsight®: Analyses of 39 genes Associated with Hereditary Cancer

RESULTS

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

SUMMARY

NEGATIVE: No Clinically Significant Variants Detected

INTERPRETATION

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Genes Analyzed (39 total): **APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, FH, FLCN, MET, MLH1, MSH2, MSH6, MUTYH, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53, TSC1, TSC2** and **VHL** (sequencing and deletion/duplication); **AXIN2, HOXB13, MBD4, MSH3, POLD1** and **POLE** (sequencing only); **EPCAM** and **GREM1** (deletion/duplication only). RNA data is routinely analyzed for use in variant interpretation for all genes.

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- CancerNext® +RNAinsight® (Product Code 8824-R)

ASSAY INFORMATION

General methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing (NGS). Variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants are verified by Sanger sequencing. Gross deletion/duplication analysis is performed using a customized pipeline using a combination of third-party coverage-based tools and custom methodologies with confirmatory MLPA and/or targeted chromosomal microarray. Mobile element insertions, if detected, are confirmed by PCR and Sanger sequencing and/or gel electrophoresis.

Ribonucleic acid (RNA) is isolated from the patient's specimen using standardized methodology and quantified. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR). Sequence enrichment is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing (NGS). RNA transcripts are screened and compared to a human reference pool. The presence of RNA transcripts meeting quality thresholds is incorporated as evidence for the assessment and classification of DNA variants. Any region not meeting RNA quality thresholds, including regions with chronically low expression in human peripheral lymphocytes, are excluded from analysis.

Additional methodology:

- **MSH2:** The inversion of coding exons 1-7 is detected by NGS and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis.
- **PMS2:** Gross deletions and duplications of exons 11-15 of *PMS2* are reflexed to long-range PCR and gel electrophoresis and/or sequencing to determine if the event occurs within *PMS2* or *PMS2CL*. The most likely deletion/duplication configuration that is consistent with the long-range PCR results is reported; however, rare complex rearrangements in *PMS2* and *PMS2CL* cannot be ruled out.

NCBI reference sequences: *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *AXIN2*- NM_004655.3, *BAP1*- NM_004656.2, *BARD1*- NM_000465.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDKN2A*- NM_000077.4 & NM_058195.3, *CHEK2*- NM_007194.3, *EPCAM*- NM_002354.2, *FH*- NM_000143.3, *FLCN*- NM_144997.5, *GREM1*- NM_013372.6, *HOXB13*- NM_006361.5, *MBD4*- NM_001276270.2, *MET*- NM_001127500.1, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *MUTYH*- NM_001128425.1, *NF1*- NM_000267.3, *NTHL1*- NM_002528.5, *PALB2*- NM_024675.3, *PMS2*- NM_000535.5, *POLD1*- NM_002691.2, *POLE*- NM_006231.2, *PTEN*- NM_000314.4, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *SMAD4*- NM_005359.5, *STK11*- NM_000455.4, *TP53*- NM_000546.4, *TSC1*- NM_000368.4, *TSC2*- NM_000548.3, *VHL*- NM_000551.3.

Analytical range: This test detects variants in the coding domains and well into the flanking 5' and 3' ends of the introns and untranslated regions. Unless explicitly stated, sequence and copy number variants in the promoter, non-coding exons, or 3' untranslated regions are not routinely reported.

Analytical range exceptions:

- **APC:** all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported.
- **EPCAM:** only gross deletions encompassing the 3' end of the gene are reported.
- **GREM1:** only the status of the 40kb 5'UTR gross duplication is analyzed and reported.
- **MSH3:** the polyalanine repeat region is excluded from analysis.
- **NTHL1:** only full-gene gross deletions and duplications are detected.
- **Gross deletion/duplication analysis is not performed for the following genes:** *AXIN2*, *HOXB13*, *MBD4*, *MSH3*, *POLD1*, *POLE*.

Reporting: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. In result reports, variants in the following classifications are always reported, and are based on the following definitions and clinical recommendations.

- **Pathogenic Mutation:** variants with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP):** variants with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS):** variants with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the

Family Studies Program may be recommended. Medical management to be based on personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 base pairs from the splice junction when detected.

Variants of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included in results. These include findings classified as "likely benign" and "benign" variants.

RNA transcripts derived from genes with limited gene-disease validity or with an inconsistent mechanism of disease do not routinely contribute to variant interpretation.

All results, including those from prior genetic testing for themselves and/or family members, will be reported as described above.

Gender identity (if provided) is not used in the interpretation of results, and sex assigned at birth is used in the interpretation of results only when necessary. Currently, there are insufficient data to determine specific cancer risk adjustments for transgender, nonbinary, or intersex individuals.

Assay Information Continued on Next Page

ASSAY INFORMATION (Supplement to Test Results - Continued)

Resources: The following references are frequently used in variant analysis and classification for observed genetic alterations.

1. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2015 May;17(5):405-23.
2. Ambry Genetics Variant Classification Scheme. <http://www.ambrygen.com/variant-classification>.
3. Grantham R. Amino acid difference formula to help explain protein evolution. *Science*. 1974;185(4151):862-864. PMID: 4843792.
4. Stenson PD et al. The Human Gene Mutation Database: 2008 update. *Genome Med*. 2009 Jan 22;1(1):13. doi: 10.1186/gm13. PMID: 19348700; PMCID: PMC2651586.
5. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
6. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat*. 2017 Mar;38(3):243-251. PMID: 27995669.
7. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: <http://gnomad.broadinstitute.org>.
8. Tavtigian SV et al. Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med*. 2018 Sep;20(9):1054-1060. PubMed PMID: 29300386
9. Karczewski KJ et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020 May;581(7809):434-443. PMID: 32461654.
10. Collins RL et al. A structural variation reference for medical and population genetics. *Nature*. 2020 May;581(7809):444-451. PMID: 32461652.
11. Jaganathan K et al. Predicting Splicing from Primary Sequence with Deep Learning. *Cell*. 2019 Jan 24; 176(3):535-548.e24. PMID: 30661751.
12. Tavtigian SV et al. Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines. *Hum Mutat* 2020 Oct;41(10):1734-1737. PMID:32720330.
13. Pejaver et al. Calibration of computational tools for missense variant pathogenicity classification and ClinGen recommendation for PP3/BP4 criteria. *Am J Hum Genet*. 2022 Dec 1;109(12):2163-2177. PMID:36413997.
14. Walker LC et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: Recommendations from the ClinGen SVI Splicing Subgroup. *Am J Hum Genet* 2023. PMID: 37352859.
15. Whiffin N et al. Using high-resolution variant frequencies to empower clinical genome interpretation. *Genet Med*. 2017 Oct;19(10):1151-1158. PMID: 28518168.

Disclaimer: This test was developed, and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. This test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels, and gross deletions/duplications. Unless otherwise noted in the methodology section above, this test is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, mobile element insertions, and other unknown abnormalities. The pattern of mutation types varies by gene, and this test detects a high but variable percentage of known and unknown mutations of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting ~99.9% of described mutations in the genes represented on the test, listed above (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Mutations in other genes or the regions not analyzed by this test can also give rise to similar clinical conditions. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, germline or somatic mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, active hematologic disease, a history of allogeneic bone marrow or peripheral stem cell transplant, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.

Understanding Your Negative Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS

RESULT	The test result was negative, which means that the test did not find any pathogenic mutations (known to be disease-causing) or likely pathogenic variants (likely to be disease-causing). You may still have a pathogenic mutation or likely pathogenic variant in a gene that was not included in this test. It is also possible that your condition may not be caused by a change in a gene.
CANCER RISK	Even though your genetic test result was negative, you and your relatives may still have an increased risk of developing cancer based on other factors, including your medical and/or family history. It is important to discuss these risk factors with your healthcare provider.
WHAT YOU CAN DO	Risk management decisions are very personal, and depend on many factors. It is important to discuss these options with your healthcare provider and decide on a plan that works for you.
FAMILY	Depending on their medical and/or family history, your relatives may still have an increased risk of developing cancer and may be eligible for genetic testing and/or increased cancer screening. It is important that they discuss their options with their healthcare provider and decide on a plan that works for them.
RESOURCES	<ul style="list-style-type: none">• American Cancer Society cancer.org• National Society of Genetic Counselors nsgc.org• Canadian Association of Genetic Counsellors cagc-accg.ca

Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your genetic test result, medical recommendations, genetic testing options, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.