Ambry Genetics®

SAMPLE REPORT

Ordered By	Contact ID:405956	Org ID:249	Patient Name:	**Last, First**	
Medical Professional:	Sample Doctor, A		Accession #:	**00-332049**	Specimen #: 44-55-66
Client:	Sample Organization (00403)		AP2 Order #:	205725	Specimen: Blood EDTA (Purple top)
Additional Authorized Recipient:			Birthdate:	01/01/1980	Sex at Birth: F
Sample Genetic Counselor, MS			MRN #:	####	Collected: 05/18/2018
			Indication:	Diagnostic/Family History	Received: 05/19/2018
					Test Started: 02/27/2024

CancerNext®: Analyses of 34 Genes Associated with Hereditary Cancer

RESULTS

CHEK2 Variant, Unknown Significance: p.E64K

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- **No known clinically actionable alterations were detected.**
- One variant of unknown significance was detected in the *CHEK2* gene.
- **Risk Estimate:** should be based on clinical and family history, as the clinical significance of this result is unknown.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.E64K (c.190G>A) variant of unknown significance in the *CHEK2* gene, which may or may not contribute to this individual's clinical history. Refer to the supplementary pages for additional information on this variant. No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (34 total): ***APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, MLH1, MSH2, MSH6, MUTYH, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, SMARCA4, STK11*** **and** ***TP53*** **(sequencing and deletion/duplication);** ***AXIN2, HOXB13, MSH3, POLD1*** **and** ***POLE*** **(sequencing only);** ***EPCAM*** **and** ***GREM1*** **(deletion/duplication only)**.

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® (Product Code 8824)

ASSAY INFORMATION

Methodology: The **CancerNext®** test is a comprehensive screen of 34 genes associated with hereditary cancer predisposition. 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). Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. 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. The *BRCA2* Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu), and the *MSH2* coding exons 1-7 inversion are detected by NGS and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for the genes sequenced (excluding *AXIN2, HOXB13*, *MSH3*, *POLD1,* and *POLE*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. 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. Sequence analysis is based on the following NCBI reference sequences: *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *AXIN2*- NM_004655.3, *BARD1*- NM_000465.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN2A*- NM_000077.4 and NM_058195.3 (p14ARF), *CHEK2*- NM_007194.3, *DICER1*-NM_177438.2, *HOXB13*- NM_006361.5, *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, *SMARCA4*- NM_001128849.1, *STK11*- NM_000455.4, *TP53*- NM_000546.4.

Analytical Range: The **CancerNext®** test detects variants in the sequenced genes (*APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NF1, NTHL1*, *PALB2, POLD1, POLE, PMS2, PTEN, RAD51C, RAD51D, SMAD4, SMARCA4, STK11,* and *TP53)* by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all 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. For *HOXB13*, only variants impacting codon 84 are routinely reported. For *POLD1* and *POLE*, only missense variants and in-frame insertions/deletions in the exonuclease domains (codons 311-541 and 269-485, respectively) are routinely reported. The *MSH3* polyalanine repeat region is excluded from analysis. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of sequenced genes (excluding *AXIN2, HOXB13, MSH3, POLD1,* and *POLE*) as well as *GREM1* and *EPCAM*. For *GREM1,* only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported. For *NTHL1*, only full-gene gross deletions and duplications are detected. For *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.

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

- **Pathogenic Mutation**: alterations 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)**: alterations 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)**: alterations 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.

Alterations 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" alterations.

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

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

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

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. 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.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647
17. Karczewski KJ et al. *Nature*. 2020 May;581(7809):434-443. PMID: 32461654
18. Splicing Prediction: Jaganathan K et al. *Cell.* 2019 Jan 24; 176(3):535-548.e24. PMID: 30661751

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. 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 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, it is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants 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% of described mutations in the 36 genes represented on the panel (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Breast, ovarian and colon cancers are complex clinical disorders. Mutations in other genes or the regions not analyzed by this test can also give rise to clinical conditions similar to breast cancer, ovarian or colon cancer. 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 can lead to misinterpretation of patient sequence data**.** 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.

CHEK2 NM_007194 c.190G>A p.E64K

VARIANT DETAILS:

The **p.E64K** variant (also known as c.190G>A), located in coding exon 1 of the *CHEK2* gene, results from a G to A substitution at nucleotide position 190. The glutamic acid at codon 64 is replaced by lysine, an amino acid with similar properties. This alteration has been detected in multiple cohorts of breast, ovarian and prostate cancer patients (Dong X et al. *Am. J. Hum. Genet.* 2003 Feb;72:270-80; Wu X et al. *Hum. Mutat.* 2006 Aug;27:742-7; Desrichard A et al. *Breast Cancer Res.* 2011;13:R119; Tung N et al. *J. Clin. Oncol*. 2016 May;34:1460-8; Susswein LR et al. *Genet. Med*. 2016 Aug;18:823-32; Kraus C et al. *Int. J. Cancer.* 2017 Jan;140:95-102; Tsaousis GN et al. *BMC Cancer.* 2019 Jun;19:535; Tsai GJ et al. *Genet. Med.* 2019 06;21:1435-1442; Girard E et al. *Int. J. Cancer.* 2019 04;144:1962-1974). Functional studies conducted in multiple model systems have conflicting results as to the effect this protein has on substrate phosphorylation and DNA damage response (Wu X et al. *Hum. Mutat.* 2006 Aug;27:742-7; Roeb W et al. *Hum. Mol. Genet.* 2012 Jun;21:2738-44; Delimitsou A et al. *Hum. Mutat.* 2019 05;40:631-648; Kleiblova P et al. *Int. J. Cancer.* 2019 10;145:1782-1797; Boonen RACM et al. *Cancer Res*. 2022 Feb;82(4):615-631; Stolarova L et al. *Clin Cancer Res*. 2023 Aug;29(16):3037-3050). Although this variant may segregate incompletely with disease, due to high false positive rates and low true positive rates with few pedigrees, co-segregation analysis should be used with extreme caution for genes with low relative risk like *CHEK2* (Belman S et al. *Genet. Med.* 2020 Dec;22:2052-2059). This amino acid position is poorly conserved in available vertebrate species. Based on the available evidence, the clinical significance of this alteration remains unclear.

GENE INFORMATION:

The *CHEK2* gene (NM_007194.3) is located on chromosome 22q12.1, encodes the serine/threonine-protein kinase Chk2 protein, and contains 14 coding exons. Pathogenic variants in this gene have been detected in individuals with increased susceptibility to breast cancer, which is inherited in an autosomal dominant fashion. Pathogenic variants in *CHEK2* confer a significantly increased cumulative lifetime risk for female breast cancer (20-40%) and may confer an increased lifetime risk for colorectal cancer (CRC) (5-10%); this risk is demonstrated particularly in individuals with a family history of CRC (The *CHEK2* Breast Cancer Case-Control Consortium. *Am. J. Hum. Genet*. 2004 Jun;74(6):1175-82; Xiang HP et al. *Eur J Cancer.* 2011 Nov;47(17):2546-51; Näslund-Koch C et al. *J. Clin. Oncol*. 2016 Apr;34(11):1208-16; Breast Cancer Association Consortium. *N Engl J Med.* 2021 Feb 4;384(5):428-439; Koen K et al. *Hered Cancer Clin Pract* 20, 5 2022; Hu C et al. *N Engl J Med.* 2021 Feb 4;384(5):440-451; Leedom T et al. *Cancer Genet*. 2016 Sep;209(9):403-407; Liu C et al. *Asian Pac J Cancer Prev.* 2012;13(5):2051-5; Katona B et al. *Genet Med.* 2018 Nov;20(11):1324-1327). *CHEK2* pathogenic variants have also been identified in individuals with male breast cancer, thyroid cancer, renal cancer, and prostate cancer; however, current risk estimates are not clearly defined (Liu C et al. *Asian Pac J Cancer Prev.* 2012;13:2051-5; Katona B et al. *Eur J Cancer.* 2017 Sep;83:103-105.; 47:2546-2551; Naslund-Koch C et al. *J Clin Oncol*. 2016 Apr 10;34(11):1208-16; Wang Y et al. *Int J Clin Exp Med.* 2015; 8(9): 15708–15715; Koen K et al. *Hered Cancer Clin Pract.* 2022 Jan 31;20(1):5; Leedom et al.). Studies have shown that female carriers of two *CHEK2* pathogenic variants (biallelic carriers) may be diagnosed more frequently with breast cancer and/or at earlier ages; however, current data is insufficient to determine exact risk estimates compared to monoallelic carriers (Rainville I et al. *Breast Cancer Res Treat*. 2020 Apr;180(2):503-509; Bychovsky B et al. *JAMA Oncol.* 2022 Sep 22;e224071; Adank M et al. *J Med Genet.* 2011 Dec;48(12):860-3). The penetrance of *CHEK2* pathogenic variants is incomplete, and variable expressivity is observed; therefore, cancer risks and phenotype will differ based on individual and family history (Bychovsky et al. 2022; Laitman Y et al. *Fam Cancer*. 2022 Jul;21(3):305-308; Hu et al. 2021). Loss of function has been reported as the mechanism of disease for *CHEK2*-associated tumor predisposition.

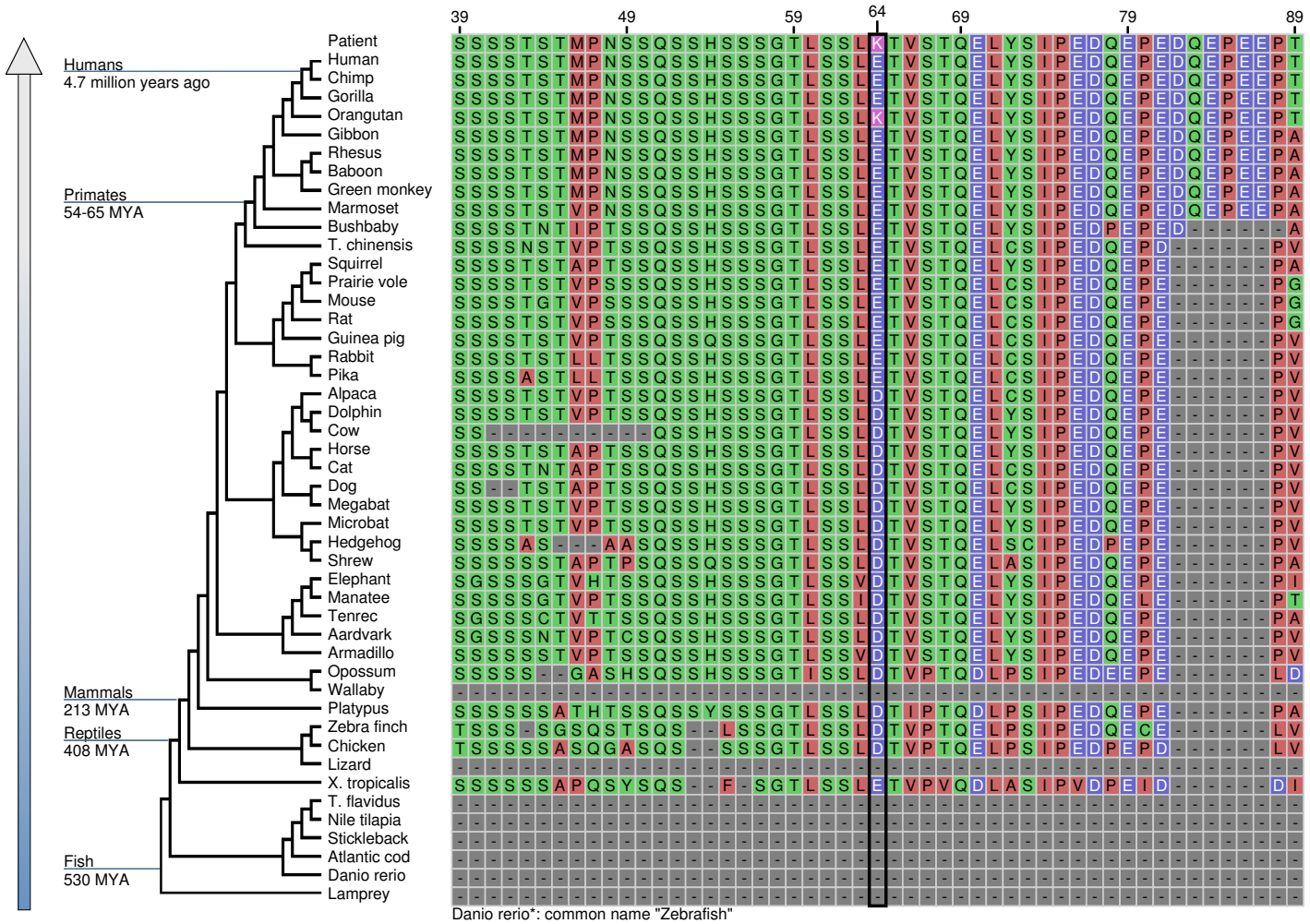
ADDITIONAL SUPPORTING INFORMATION:

Co-Segregation	Co-segregation data for this variant is currently unavailable.
Co-occurrence	No significant co-occurrence data is currently available at our laboratory.
Frequency	Internal Frequency: 0.05% (285/632000) total alleles studied.
	gnomAD: 0.016% (45/282814) total alleles studied, 0.03% (39/129148) European (non-Finnish) alleles.
Grantham Score	56 (similar amino acid substitution)
in silico	Inconclusive

CHEK2 NM_007194 c.190G>A p.E64K

Evolutionary conservation diagram: Amino Acid Alignment

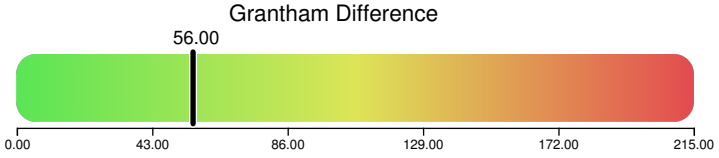
This amino acid position is poorly conserved in available vertebrate species.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Glu (E)	Lys (K)
Amino Acid Name	Glutamic acid	Lysine
Polarity/Charge	negatively charged	positively charged
pH	acidic	basic
Residue Weight	129	128
Hydrophobicity Score	-3.5	-3.9
Hydrophilicity Score	3	3
Secondary Structure Propensity	strong α former / strong β breaker	α former / β breaker



Ambry Genetics®

Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A VARIANT OF UNKNOWN SIGNIFICANCE

RESULT	The testing found one or more variants of unknown significance (VUS). There is not currently enough information available to know if the VUS identified is expected to cause an increased risk for cancer or not.
RECLASSIFICATION	Collecting information about a VUS is an ongoing process, so it is possible that your result may be better understood in the future. Ambry regularly reviews the data and published evidence about each VUS, and your healthcare provider will be notified if enough new information becomes available to reclassify your VUS. For this reason, it is recommended that you continue to follow-up with the healthcare provider that ordered your genetic testing.
CANCER RISK	Even though your genetic test result was a VUS, 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	Certain family members may be eligible for genetic testing through our family studies program. In some cases, testing family members may help add to the understanding of your result. However, not all genes are well suited for family studies testing. To determine if your VUS is eligible for family studies testing, your healthcare provider can contact FamilyStudies@ambrygen.com.
RESOURCES	• 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.

WHAT VARIANT CLASSIFICATIONS MEAN

PATHOGENIC MUTATION (POSITIVE TEST RESULT)	Contains enough evidence showing it can cause a disease
VARIANT, LIKELY PATHOGENIC (VLP, POSITIVE TEST RESULT)	Strong evidence to suggest it causes a disease
VARIANT OF UNKNOWN SIGNIFICANCE (VUS)	Limited and/or conflicting evidence to suggest it may cause a disease
VARIANT, LIKELY BENIGN (VLB, NEGATIVE TEST RESULT)	Strong evidence to suggest it does not cause a disease
BENIGN (NEGATIVE TEST RESULT)	Contains enough evidence to show it does not cause a disease

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PROMPT

Prospective Registry Of MultiPlex Testing

Opportunity to Enroll in Hereditary Cancer Research

Genetic testing can help individuals and families by giving them a clearer idea of their cancer risks. Genetic tests (called multi-gene or multiplex panels) look for changes in several different genes, all in a single test. While all of the genes on these panels have been tied to an increased risk of cancer, we understand the risks associated with some of the genes better than we understand others. One way to help improve our understanding is to enroll people with pathogenic mutations or variants of unknown significance in registries. Registries typically follow people over many years to learn more about these alterations and how they impact their health.

How can I find a research registry?

There are several hereditary cancer research registries that are studying individuals who have had multiplex panel testing. One registry that is open to individuals nationwide is PROMPT (or **P**rospective **R**egistry **O**f **M**ulti**P**lex **T**esting). PROMPT is an online registry for patients and families who have had multiplex testing and have been found to have a genetic variation which may be linked to an increased risk of cancer. PROMPT is a joint effort involving several academic medical centers and commercial laboratories, working together to learn more about the genes that are studied on multiplex panels. PROMPT will allow researchers to better understand the cancer risks associated with changes in these genes and thus provide a better understanding of the best way to take care of individuals who have such changes.

What is involved in participation?

Participation in the study simply involves completing online surveys. Additionally, the PROMPT team may reach out to you to talk about ways that you can get more involved with the research effort. Your participation will help researchers learn more and improve the ability of this genetic testing to help people.

How do I enroll?

You can learn more about or register for PROMPT by going to www.promptstudy.info or by scanning the QR code below.

Thank you again for considering taking part in PROMPT!

If you would like to read more about multiplex panels, including details about specific genes, please visit our informational website at www.promptstudy.info.