Ambry Genetics
A TEMPUS COMPANY

SAMPLE REPORT

Ordered By	Contact ID:6323407	Org ID:8141	
Medical Professional:	Unknown, Unknown, MD		
Client:	MOCKORG44 (10829)		

Patient Legal Name:	**Last, First**		
Accession #:	**01-326957**	Specimen #:	
AP2 Order #:	3105392	Specimen:	Adult Saliva (Oragene Kit)
Birthdate:	01/01/1998	Sex assigned at birth:	F
MRN #:	N/A	Collected:	05/03/2025
Indication:	Diagnostic/Family History	Received:	05/06/2025
		Test Started:	05/06/2025

CancerNext®: Analyses of 40 Genes Associated with Hereditary Cancer

RESULTS

BRCA1 Variant, Unknown Significance: p.A1142T

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- **No known clinically actionable alterations were detected.**
- One variant of unknown significance was detected in the *BRCA1* 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.A1142T (c.3424G>A) variant of unknown significance in the *BRCA1* 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 (40 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, RPS20, 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)**.

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

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.

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, *RPS20*-NM_001023.3, 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 Variant**: 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 variant carriers recommended. Previously described pathogenic variants, including intronic variants 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, Uncertain 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. Classification and interpretation of variants may change over time with accumulating evidence and scientific advancements. Updated classifications may be reported through reclassification notices; however, clients should re-contact the laboratory or visit ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) for the most up to date information regarding the

current interpretation of results.

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 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 (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.

BRCA1 NM_007294 c.3424G>A p.A1142T

VARIANT DETAILS:

The **p.A1142T** variant (also known as c.3424G>A), located in coding exon 9 of the *BRCA1* gene, results from a G to A substitution at nucleotide position 3424. The alanine at codon 1142 is replaced by threonine, an amino acid with similar properties. This amino acid position is conserved. In addition, this alteration is predicted to be tolerated by *in silico* analysis. Based on the available evidence, the clinical significance of this variant remains unclear.

FAMILY STUDIES PROGRAM:

Ambry Genetics offers complimentary genetic studies for variants of unknown significance (VUSs) meeting specific criteria in appropriate family members. Review of clinical information is required. Additional information, application instructions and required forms, and patient education materials are available at http://ambrygen.com/family-studies-program. For additional information, please email us at GeneticCounselor@ambrygen.com or call 949-900-5500 and ask to speak with a genetic counselor.

Please note that the classification of variants may change over time as additional information becomes available. Alerts are disseminated via fax and/or AmbryPort email to clinicians upon clinically relevant variant reclassifications. If no updates are received, clinicians are encouraged to contact the laboratory at 949-900-5500 once a year to review the status of previously reported variants.

GENE INFORMATION:

The *BRCA1* gene (NM_007294.3) is located on chromosome 17q21.31, encodes the breast cancer type 1 susceptibility protein, and contains 22 coding exons. Pathogenic variants in this gene are known to cause *BRCA1*-related cancer predisposition, which is inherited in an autosomal dominant fashion, and *BRCA1*-related Fanconi anemia, which is inherited in an autosomal recessive fashion. *BRCA1*-related cancer predisposition is characterized by a significantly increased cumulative lifetime risk for female breast cancer (60-72%), male breast cancer (up to 1.2%), epithelial ovarian cancer (39-58%), pancreatic cancer (3-5%), and prostate cancer (7-26%). *BRCA1*-related cancer predisposition is also associated with a contralateral female breast cancer risk of up to 40% within 20 years of initial breast cancer diagnosis with no intervention; however, this risk is age-dependent and more significant with earlier age (prior to age 40) of first breast cancer diagnosis (Kuchenbaecker K et al. *JAMA*. 2017 Jun 20;317(23):2402-2416; Hu C et al. *J Natl Cancer Inst*. 2020 Dec 14;112(12):1231-124; Breast Cancer Association Consortium. N Engl J Med. 2021;384:428-439; Hu C et al. *N Engl J Med*. 2021 Feb 4; 384(5): 440–451; Tai Y et al. *J Natl Cancer Inst*. 2007 Dec 5;99(23):1811-4; Chen J et al. *JNCI Cancer Spectr*. 2020 Apr 23;4(4):pkaa029; Chaffee K et al. *Genet Med*. 2018 Jan;20(1):119-127; Hu C et al. *JAMA*. 2018 Jun 19;319(23):2401-2409). Penetrance in individuals with *BRCA1*-related cancer predisposition is incomplete and variable expressivity is observed; therefore, cancer risks will differ based on individual and family history. Published evidence suggests that both germline and somatic alterations in the *BRCA1* gene may predict sensitivity to chemotherapy agents that induce DNA damage as well as to poly(ADP-ribose) polymerase (PARP) inhibitors (Kim G et al. *Clin Cancer Res*. 2015 Oct 1;21(19):4257-61; Balasubramaniam S et al. *Clin. Cancer Res*., 2017 Dec;23:7165-7170).Loss of function has been reported as the mechanism of disease for *BRCA1*-related cancer predisposition. *BRCA1*-related Fanconi anemia is characterized by progressive bone marrow failure, adult onset aplastic anemia, pre- and postnatal growth deficiency, abnormal skin pigmentation, characteristic skeletal malformations, and impaired endocrine functioning. *BRCA1*-related Fanconi anemia can be established in a patient following cytogenetic testing of patient lymphocytes that demonstrate increased chromosomal breakage and radial forms following diepoxybutane and mitomycin C exposure (Mehta P et al. *Fanconi Anemia*. 2002 Feb 14 [updated 2021 Jun 3]. In: GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022). Individuals with *BRCA1*-related Fanconi anemia are at an increased risk of malignancies with highest risk of acute myelogenous leukemia, early-onset solid tumors including head and neck squamous cell carcinoma, and non-melanoma skin cancer (García-de-Teresa B et al. *Genes (Basel)*. 2020 Dec 21;11(12):1528., 2020). Individuals of reproductive age are at 25% risk of having a child with Fanconi anemia with each pregnancy when both biological parents have a pathogenic variant in *BRCA1*. Biallelic loss of function has been reported as the mechanism of disease for *BRCA1*-related Fanconi anemia.

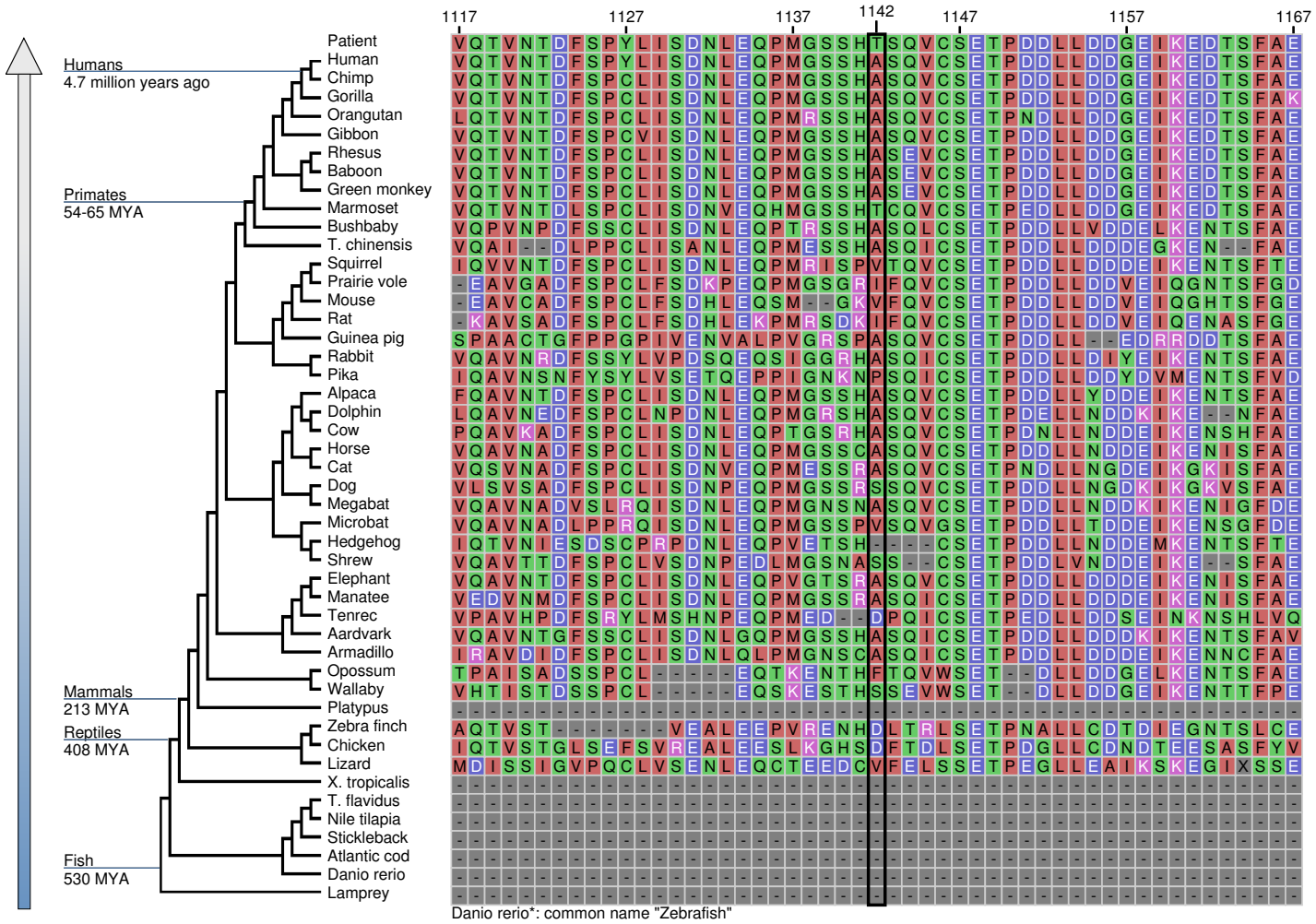
ADDITIONAL SUPPORTING INFORMATION:

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	No population frequency information could be found.
Grantham Score	58 (similar amino acid substitution)
in silico	Tolerated

BRCA1 NM_007294 c.3424G>A p.A1142T

Evolutionary conservation diagram: Amino Acid Alignment

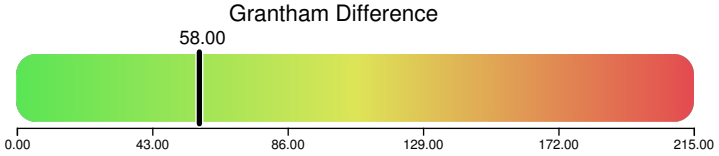
This amino acid position is conserved.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Ala (A)	Thr (T)
Amino Acid Name	Alanine	Threonine
Polarity/Charge	non-polar	polar
pH	neutral	neutral
Residue Weight	71	101
Hydrophobicity Score	1.8	-0.7
Hydrophilicity Score	-0.5	-0.4
Secondary Structure Propensity	strong α former / β indifferent	α indifferent / β former



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.