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SAMPLE REPORT

Ordered By	Contact ID:405956	Org ID:249	Patient Name:	**Last, First**	
Physician:	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	Gender: F
Sample Genetic Counselor MS, CGC			MRN #:	####	Collected: 05/18/2018
			Indication:	Diagnostic/Family History	Received: 05/19/2018

BRCA1/2 Analyses with CancerNext®

RESULTS

CHEK2 Variant, Unknown Significance: p.S39P

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 testing for variants of unknown significance (VUSs) in family members may be pursued to help clarify VUS significance, but cannot be used to identify at-risk individuals at this time.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.S39P (c.115T>C) 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 (36 total): ***APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11*** **and** ***TP53*** **(sequencing and deletion/duplication); *HOXB13, 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.

- BRCA1/2 seq and del/dup (Product Code 8838)
- CancerNext® (Product Code 8824)

ASSAY INFORMATION

General Information: Cancer is a complex, multifactorial disease diagnosed in approximately 1 out of every 2 men and 1 out of every 3 women over the course of a lifetime. Mutations in cancer predisposition genes appear to be responsible for between 5-10% of cancer diagnoses.

Methodology: The **CancerNext®** test is a comprehensive screen of 36 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. 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 next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for the genes sequenced (excluding *HOXB13*, *POLD1, POLE, PMS2*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Gross deletion/duplication analysis of *PMS2* is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. 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, *MUTYH*- NM_001128425.1, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *NBN*- NM_002485.4, *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, *RECQL*- NM_002907.3, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *STK11*- NM_000455.4, *TP53*- NM_000546.4.

Analytical Range: The **CancerNext®** test targets detection of DNA sequence mutations in the sequenced genes (*APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1*, *PALB2, POLD1, POLE, PMS2, PTEN, RAD51C, RAD51D, RECQL, 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. 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. For *RECQL*, only missense variants in the helicase and RCQ domains (codons 63-592) and exonic truncating variants 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 *HOXB13, 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 recommended. Medical management to be based personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs 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 on results reports. These include findings classified as "likely benign" and "benign" alterations.

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

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, low-level mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, presence of pre-malignant or malignant cells in the sample, 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.115T>C p.S39P

VARIANT DETAILS:

The **p.S39P** variant (also known as c.115T>C), located in coding exon 1 of the *CHEK2* gene, results from a T to C substitution at nucleotide position 115. The serine at codon 39 is replaced by proline, an amino acid with similar properties. This amino acid position is well conserved in available vertebrate species. In addition, this alteration is predicted to be tolerated by *in silico* analysis. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear.

GENE INFORMATION:

The *CHEK2* gene (NM_007194.3) is involved in the Fanconi anemia (FA)–BRCA pathway, which is critical for DNA repair by homologous recombination and the maintenance of genomic stability, and interacts *in vivo* with ATM, BRCA1, and p53. Studies indicate that mutations in the *CHEK2* gene may confer an increased risk of developing many types of cancer including breast, prostate, colon, thyroid, ovarian, and kidney (Cybulski C et al. *Am J Hum Genet.* 2004;75:1131-1135; Xiang HP et al. *Eur J Cancer.* 2011 Nov;47(17):2546-51; Walsh T et al. *PNAS.* 2011;108(44):10832-37; Näslund-Koch C et al. *J. Clin. Oncol.* 2016 Apr;34(11):1208-16; Pritchard CC et al. *N. Engl. J. Med.* 2016 Aug;375(5):443-53). A female carrier of a *CHEK2* mutation has approximately a 2 fold increase in lifetime breast cancer risk and a female carrier of *CHEK2* homozygous mutations has a 4-6 fold increase in lifetime breast cancer risk; however this risk may be modified by breast cancer family history (*CHEK2* Breast Cancer Case-Control Consortium. *Am J Hum Genet.* 2004 Jun;74(6):1175-82; Walsh T et al. *JAMA.* 2006;295(12):1379-1388; Cybulski C et al. *J CLin Oncol.* 2011 Oct 1;29(28):3747-52; Adank et al. *J Med Genet.* 2011 Dec;48(12):860-3). Increased risks of male breast cancer have also been reported (Wasielewski et al. *Breast Cancer Res Treat.* 2009 Jul;116(2):397-400).

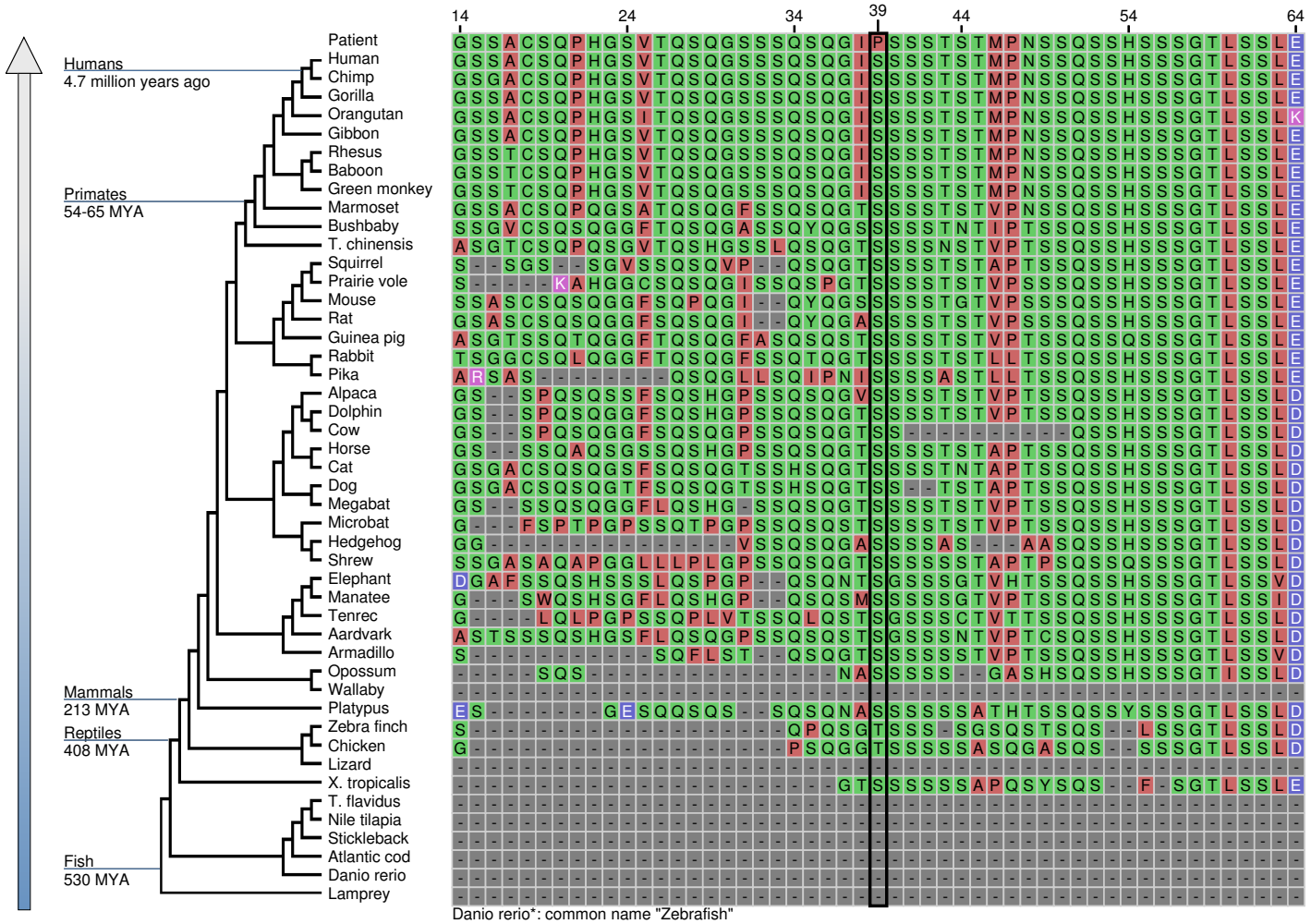
ADDITIONAL SUPPORTING INFORMATION:

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: <0.01% (3/398000) total alleles studied.
	No population frequency information could be found.
Grantham Score	74 (similar amino acid substitution)
in silico	Tolerated

CHEK2 NM_007194 c.115T>C p.S39P

Evolutionary conservation diagram: Amino Acid Alignment

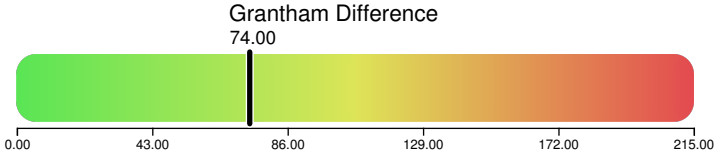
This amino acid position is well conserved in available vertebrate species.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Ser (S)	Pro (P)
Amino Acid Name	Serine	Proline
Polarity/Charge	polar	non-polar
pH	neutral	neutral
Residue Weight	87	97
Hydrophobicity Score	-0.8	-1.6
Hydrophilicity Score	0.3	0
Secondary Structure Propensity	α indifferent / β breaker	strong α breaker / strong β breaker



Ambry Genetics®

Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A **VARIANT OF UNKNOWN SIGNIFICANCE**

Result	**VUS**	Your testing found at least one variant of unknown significance (VUS) in a gene tested. A VUS is a change in a gene from what we expect to see, but we do not know if it causes an increased risk for cancer or not.
Reclassification	**POSSIBLE**	Collecting information about a VUS is an ongoing process, so it is possible that your result may be better understood in the future. The healthcare provider that ordered your test will be notified if new information becomes available about your VUS.
Cancer Risk	**VARIES**	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. Your healthcare provider can help you learn more about this.
Risk Management	**VARIES**	Risk management decisions are very personal and depend on many factors. Talk to your doctor about which, if any, options may be right for you.
Family Members	**POSSIBLE FURTHER TESTING**	Certain family members may be eligible for genetic testing through our Family Studies Program. In some cases, this may help add to the understanding of your result. If you and your relatives are interested in this, please speak to your healthcare provider about it.
Next Steps	**DISCUSS**	It is recommended that you stay in contact with your healthcare provider on a regular basis for possible new information about your result.
Reach Out	**RESOURCES**	• Ambry's Hereditary Cancer Site for Families patients.ambrygen.com/cancer • American Cancer Society cancer.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.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.

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Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A **VARIANT OF UNKNOWN SIGNIFICANCE**

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 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

1. **Does finding a VUS on genetic testing change medical management recommendations?**

   VUS by definition have not been proven to increase an individual's risk for disease or to be the cause of the disease within a family. Medical recommendations should be based on personal and/or family history of a specific disease.

2. **What percentage of VUS are reclassified?**

   Of the VUS that are reclassified, the vast majority will be reclassified to VLB or benign, although many VUS will not be reclassified at all due to lack of additional information. Only a small percentage of VUS will ultimately be reclassified to VLP or pathogenic.

3. **How long does it take to reclassify a VUS?**

   This depends upon several factors:
   - How often the VUS is found in individuals (rare variants may take longer to reclassify)
   - How common the disease is in the general population and how strongly the gene has been linked to the disease
   - Participation of certain families with the VUS in our Family Studies Program
   - Eligibility for additional specialized testing performed by Ambry's Translational Genomics (ATG) laboratory
   - Amount of active research taking place on a particular gene or VUS

4. **Who is notified if a VUS gets reclassified?**

   When enough evidence becomes available to cause a significant change, Ambry will make every attempt to send reclassification alerts for a VUS that gets reclassified to the healthcare provider.

5. **What is Ambry's Family Studies Program, and is it worth participating in it?**

   Our Family Studies Program and ATG lab include follow-up testing for you or certain family members after a VUS has been found. These studies can be worthwhile if many family members (especially those with the disease) are willing to participate. For more information, please visit our website for the Family Studies Program or ATG lab.

6. **Does Ambry perform family studies for VUS in all genes?**

   Not all genes are well suited for family studies. To find out if the VUS found is eligible for family studies contact FamilyStudies@ambrygen.com

7. **How often does Ambry check to see if there is new information about a VUS?**

   Ambry regularly assesses the data and emerging evidence related to a specific variant. Healthcare providers are welcome to contact Ambry Genetics at +1.866.262.7943 on a yearly basis to request the most current assessment of a particular variant.

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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 multi-gene panel testing. One registry that is open to individuals nationwide is PROMPT (or Prospective Registry Of MultiPlex Testing). PROMPT is an online registry for patients and families who have had multi-gene 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 multi-gene 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. You can take part without providing any personal information to the PROMPT study. But, if you are interested, the PROMPT team will reach out to you to talk about ways that you can get more involved with the research effort. Either way, 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.org
or by scanning the QR code to the right.

Thank you again for considering taking part in PROMPT!

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

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