Ambry Genetics®
A TEMPUS COMPANY

SAMPLE REPORT

Ordered By	Contact ID:8021841	Org ID:8141	Patient Legal Name: **Last, First**		
Medical Professional:	Unknown, Unknown, MD		Accession #: **01-495602**	Specimen #:	
Client:	MOCKORG44 (10829)		AP2 Order #: 3774878	Specimen: Blood EDTA (Purple top)	
			Birthdate: 01/01/1998	Sex assigned at birth: F	
			MRN #: N/A	Collected: 07/07/2026	
			Indication: Diagnostic/Family History	Received: 07/07/2026	
				Test Started: 07/07/2026	

CancerNext®: Analyses of 41 Genes Associated with Hereditary Cancer

RESULTS

MSH3 Variant, Unknown Significance: p.D868V

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- **No known clinically actionable alterations were detected.**
- One variant of unknown significance was detected in the *MSH3* 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.D868V (c.2603A>T) variant of unknown significance in the *MSH3* 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 (41 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, MLH3, 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 followed by a confirmatory orthogonal method, as needed. 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, *MLH3*- NM_001040108.1, *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 limited to the 3' end of the gene are reported.
- ***GREM1***: only the status of the 40kb 5'UTR gross duplication is routinely 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, MLH3, 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.

MSH3 NM_002439 c.2603A>T p.D868V

VARIANT DETAILS:

The **p.D868V** variant (also known as c.2603A>T), located in coding exon 19 of the *MSH3* gene, results from an A to T substitution at nucleotide position 2603. The aspartic acid at codon 868 is replaced by valine, an amino acid with highly dissimilar properties. This amino acid position is highly conserved in available vertebrate species. In addition, this alteration is predicted to be deleterious by *in silico* analysis. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear.

GENE INFORMATION:

The *MSH3* gene (NM_002439.3) is located on chromosome 5q14.1, encodes DNA mismatch repair protein Msh3, and contains 24 coding exons. Pathogenic variants in this gene have been detected in individuals with increased susceptibility to adenomatous polyposis and colorectal cancer, which is inherited in an autosomal recessive fashion. *MSH3*-associated polyposis is characterized by the presence of colorectal adenomas and/or colorectal cancer; however, current data is insufficient to determine the penetrance associated with *MSH3*-related polyposis and/or if there is risk for extracolonic neoplasms (Adam R et al. *Am J Hum Genet*. 2016 Aug 4;99(2):337-51). There is insufficient evidence to suggest an increased cancer risk above that of the general population for monoallelic carriers of pathogenic *MSH3* variants. Individuals of reproductive age are at 25% risk of having a child with *MSH3*-related polyposis with each pregnancy when both biological parents have a pathogenic variant in *MSH3*. Biallelic loss of function has been reported as the mechanism of disease for autosomal recessive *MSH3*-related polyposis.

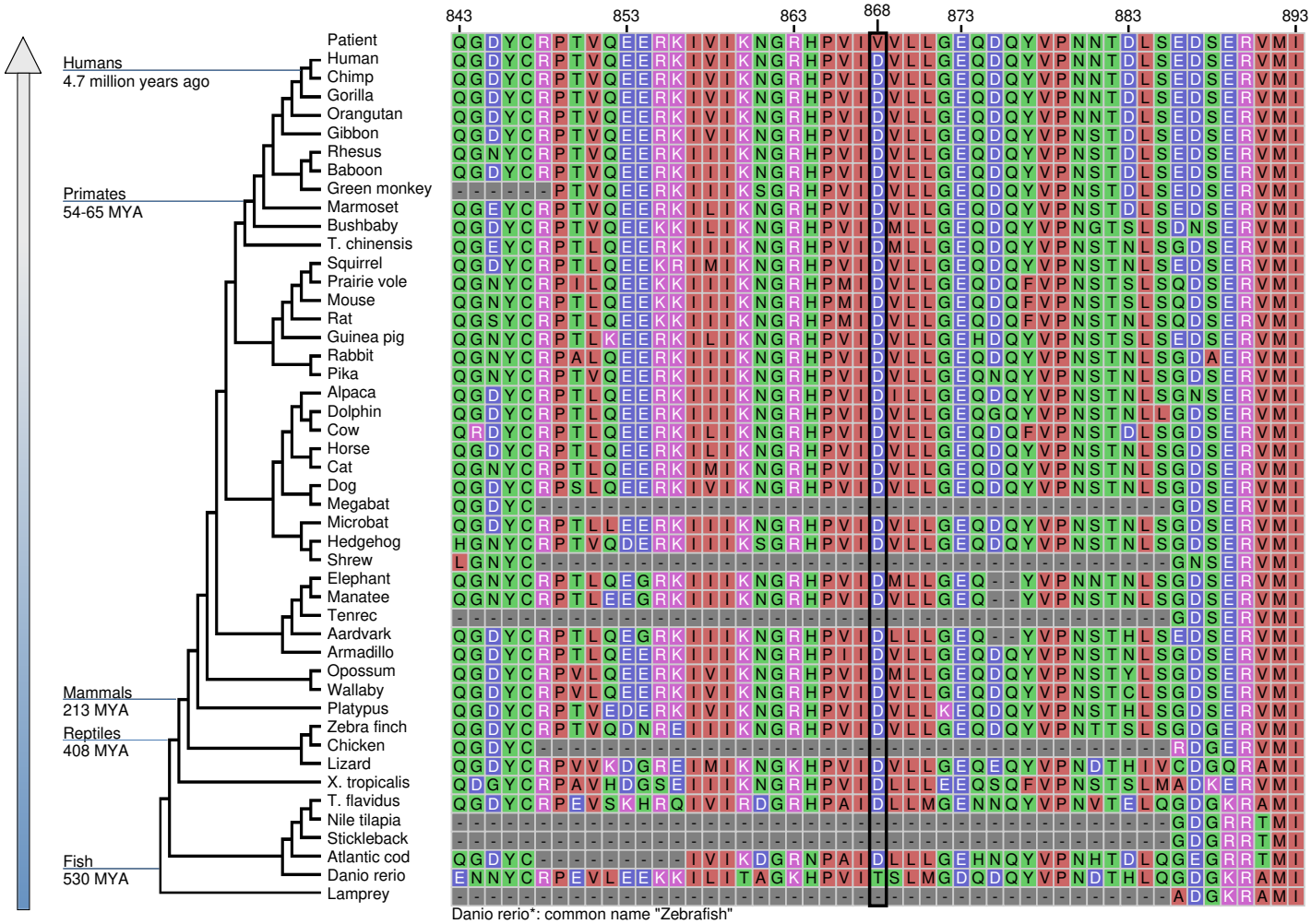
ADDITIONAL SUPPORTING INFORMATION:

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	gnomAD: <0.01% (3/251184) total alleles studied, 0.01% (2/16238) African alleles.
Grantham Score	152 (highly dissimilar amino acid substitution)
in silico	Deleterious

MSH3 NM_002439 c.2603A>T p.D868V

Evolutionary conservation diagram: Amino Acid Alignment

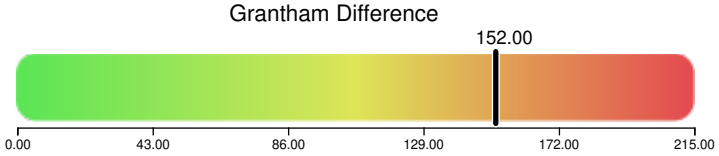
This amino acid position is highly conserved in available vertebrate species.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Asp (D)	Val (V)
Amino Acid Name	Aspartic acid	Valine
Polarity/Charge	negatively charged	non-polar
pH	acidic	neutral
Residue Weight	115	99
Hydrophobicity Score	-3.5	4.2
Hydrophilicity Score	3	-1.5
Secondary Structure Propensity	weak α former / strong β breaker	α former / strong β former



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Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A VARIANT OF UNCERTAIN SIGNIFICANCE

RESULT	The testing found one or more variants of uncertain 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

WHAT VARIANT CLASSIFICATIONS MEAN

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

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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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?

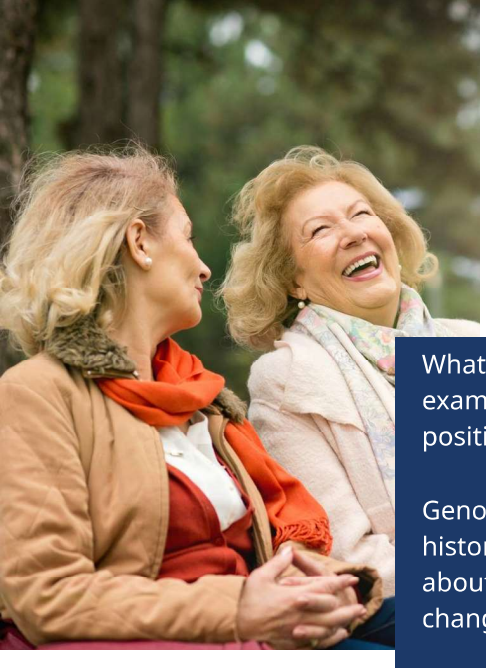
Participation in the study simply involves completing online surveys. Additionally, the PROMPT team may reach out to you to talk about ways that you can get more involved with the research effort. Your participation will help researchers learn more and improve the ability of this genetic testing to help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

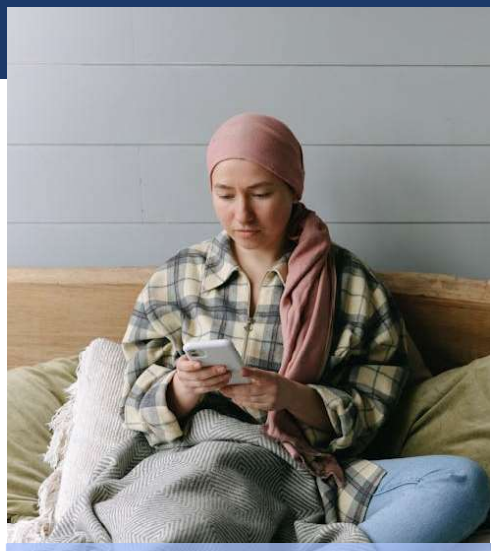
If you would like to read more about multiplex panels, including details about specific genes, please visit our informational website at www.promptstudy.info.



GenomeConnect
The ClinGen Patient Portal

What we know about genetic changes (also called variants) can change over time. For example, a result that is classified as uncertain (called a VUS) might later be changed to a positive or negative result.

GenomeConnect is a registry that helps you share your genetic test results and health history. Sharing your genetic changes and health history helps scientists learn more about genes and health and can help identify changes in variant classification over time.



Who can participate in GenomeConnect?

Anyone who has had genetic testing can join GenomeConnect. It doesn't matter what your results were—positive, negative, or uncertain (VUS). People with results in any gene can take part. GenomeConnect is available in English and Spanish.

Why join GenomeConnect?

Sometimes, when a lab finds a genetic change, they don't know what it means for health. These are called "uncertain" or "VUS" results. Even when a change is well understood, more information can help scientists learn more about the condition and how to treat it.

By joining GenomeConnect, you can securely share your genetic test results and health history with databases like ClinVar and with experts from ClinGen. ClinGen has a focus in cancer including through expert panels working to better understand genetic changes that increase cancer risk.

The more people who share their information, the more scientists can learn about how genes and genetic variants affect health and, ultimately, how best to care for patients.

By participating in GenomeConnect, you can:

- Share your information securely and safely.
- Help **increase our understanding of genetics and health** that might help you and other families.
- Have the option to **receive genetic result updates** from GenomeConnect. This can be particularly helpful for individuals with uncertain (VUS) results.
- Learn about other relevant **research opportunities.**
- **Connect with other participants** with similar results.

What are the steps to participate in GenomeConnect?

Participation is simple

- Sign up and consent www.genomeconnect.org
- Share a copy your genetic test results (no new sample needed)
- Fill out surveys to share your health history
- Update your information once a year

"It's just reassuring, really comforting to get updates and that I don't have to worry about it and just to know that the genetic industry or the genetic world, there is someone that we can rely on."

- GenomeConnect Participant discussing the option to receive genetic result updates

Learn More and Register:
www.genomeconnect.org

Questions:
info@genomeconnect.org

GenomeConnect is a project of the Clinical Genome Resource (ClinGen), an NIH funded research project.

ClinGen
Clinical Genome Resource

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