

Ordered By	Contact ID:6323418	Org ID:8141	Patient Legal Name: Last, First	
Medical Professional:	Unknown, Unknown, MD		Accession #: 01-326963	Specimen #:
Client:	MOCKORG44 (10829)		AP2 Order #: 3105398	Specimen: Blood EDTA (Purple top)
			Birthdate: 01/01/2004	Sex assigned at birth: M
			MRN #: N/A	Collected: 05/03/2025
			Indication: Diagnostic/Family History	Received: 05/06/2025
				Test Started: 05/06/2025

ColoNext®: Analyses of Genes Associated with Hereditary Colon Cancer (26 genes)

RESULTS

RPS20 Variant, Likely Pathogenic: **c.3+1G>A**

SUMMARY

POSITIVE: Likely Pathogenic Variant Detected

INTERPRETATION

- This individual is heterozygous for the **c.3+1G>A** likely pathogenic variant in the *RPS20* gene.
- This result is consistent with a diagnosis of *RPS20*-related colorectal cancer predisposition.
- **Risk estimate:** increased risk for colorectal cancer.
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for likely pathogenic variants (VLPs) in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (26 total): **APC, ATM, BMPR1A, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, NTHL1, PMS2, PTEN, RPS20, SMAD4, STK11 and TP53 (sequencing and deletion/duplication); AXIN2, CTNNA1, MBD4, MLH3, MSH3, POLD1, POLE and RNF43 (sequencing only); EPCAM and GREM1 (deletion/duplication only).**

RPS20 Additional Information

The **c.3+1G>A** intronic variant results from a G to A substitution one nucleotide after coding exon 1 of the *RPS20* gene. In silico splice site analysis predicts that this alteration will weaken the native splice donor site; however, direct evidence is insufficient at this time (Ambry internal data). The stop codon in the predicted resulting transcript occurs in the 5' end of the *RPS20* gene. As such, this alteration may escape nonsense-mediated mRNA decay and/or be prone to rescue by reinitiation (Rivas et al. *Science*. 2015 May 8;348(6235):666-9; Lindeboom et al. *Nat Genet*. 2016 Oct;48(10):1112-8; Rhee et al. *Sci Rep*. 2017 May 10;7(1):1653). The exact functional effect of this alteration is unknown; however, a significant portion of the protein is affected (Ambry internal data). This variant is considered to be rare based on population cohorts in the Genome Aggregation Database (gnomAD). This nucleotide position is highly conserved in available vertebrate species. Based on the majority of available evidence to date, this variant is likely to be pathogenic.

The *RPS20* gene (NM_001023.3) is located on chromosome 8q12.1, encodes the small ribosomal subunit protein uS10, and contains 4 coding exons. Pathogenic variants in this gene are known to cause *RPS20*-related colorectal cancer (CRC) predisposition, which is inherited in an autosomal dominant fashion. *RPS20*-related CRC is characterized by a significantly increased risk for CRC. Penetrance data is currently limited, but is suggested to be high; cancer risks will differ based on individual and family history (Nieminen TT, et al. (2014) *Gastroenterology* 147(3):595-598.e5; Broderick P, et al. (2017) *Gastroenterology* 152(1):75-77.e4; Thompson BA, et al. (2020) *Clin Genet* 97(6):943-944; Djursby M, et al. (2020) *Front Genet* 11(0):566266; Herrera-Mullar J, et al. (2025) *Gen in Med Open* 3:2 [abstract]). Variants that lead to premature truncation have been reported as disease-causing for *RPS20*-related colorectal cancer predisposition.

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.

- ColoNext® (Product Code 8821)

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, *BMPR1A*- NM_004329.2, *CDH1*- NM_004360.3, *CHEK2*- NM_007194.3, *CTNNA1*- NM_001903.2, *EPCAM*- NM_002354.2, *GREM1*- NM_013372.6, *MBD4*- NM_001276270.2, *MLH1*- NM_000249.3, *MLH3*- NM_001040108.1, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *MUTYH*- NM_001128425.1, *NTHL1*- NM_002528.5, *PMS2*- NM_000535.5, *POLD1*- NM_002691.2, *POLE*- NM_006231.2, *PTEN*- NM_000314.4, *RNF43*- NM_017763.4, *RPS20*- NM_001023.3, *SMAD4*- NM_005359.5, *STK11*- NM_000455.4, *TP53*- NM_000546.4.

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*, *CTNNA1*, *MBD4*, *MLH3*, *MSH3*, *POLD1*, *POLE*, *RNF43*.

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.

Clinician Management Resource for *RPS20*

This overview of clinical management guidelines is based on this patient's positive test result for a pathogenic or likely pathogenic variant in the *RPS20* gene. Unless otherwise stated, medical management guidelines used here are limited to those issued by the National Comprehensive Cancer Network® (NCCN®)¹ in the U.S. Please consult the referenced guideline for complete details and further information.

Clinical correlation with the patient's past medical history, treatments, surgeries and family history may lead to changes in clinical management decisions; therefore, other management recommendations may be considered. Genetic testing results and medical society guidelines help inform medical management decisions but do not constitute formal recommendations. Discussions of medical management decisions and individualized treatment plans should be made in consultation between each patient and his or her healthcare provider, and may change over time.

SCREENING CONSIDERATIONS ¹	AGE TO START	FREQUENCY
Colorectal Cancer*		
Colonoscopy	20 years old (or one year after transplant if the patient had a hematopoietic cell transplant prior to age 20)	Every 5 years

* Given the early ages of CRC in the *RPS20* families, it is recommended that individuals with pathogenic variants in *RPS20* follow colorectal cancer surveillance recommendations for Diamond-Blackfan anemia, though the link between pathogenic variants in *RPS20* and Diamond-Blackfan anemia is uncertain at present.

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric. v3.2024. © National Comprehensive Cancer Network, Inc. 2024. All rights reserved. Accessed October 31, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

Understanding Your Positive *RPS20* Genetic Test Result

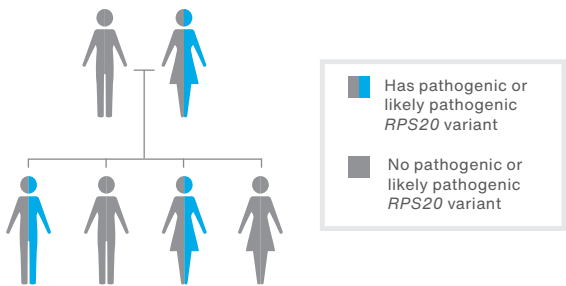
INFORMATION FOR PATIENTS WITH A PATHOGENIC OR LIKELY PATHOGENIC VARIANT

4 Things To Know

1	Result	Your testing shows that you have a pathogenic or likely pathogenic variant in the <i>RPS20</i> gene.
2	Cancer risks	You have an increased chance to develop colorectal cancer.
3	What you can do	Risk management decisions are very personal. There are options to detect cancer early or lower the risk to develop cancer. It is important to discuss these options with your healthcare provider and decide on a plan that works for you.
4	Family	Family members may also be at risk – they can be tested for the pathogenic or likely pathogenic <i>RPS20</i> variant that was identified in you. It is recommended that you share this information with family members so they can learn more and discuss this with their healthcare providers.

RPS20 in the family

There is a 50/50 random chance to pass on the pathogenic or likely pathogenic *RPS20* variant to each of your children.



RESOURCES

- AliveAndKickn (Patient Advocacy Group) aliveandkickn.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 *RPS20* result, medical recommendations, 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.



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.

Opportunity to connect and help prevent cancer in your family

Did you recently have genetic testing for a cancer gene variant (or mutation) known to be in your family? Questions such as “Where did this variant come from?” or “What can I do to help others in my family?” are common. ConnectMyVariant can help!

ConnectMyVariant provides resources for people who want help talking with relatives about cancer risk or finding new relatives who might be at risk to help them get genetic testing and prevent cancer.

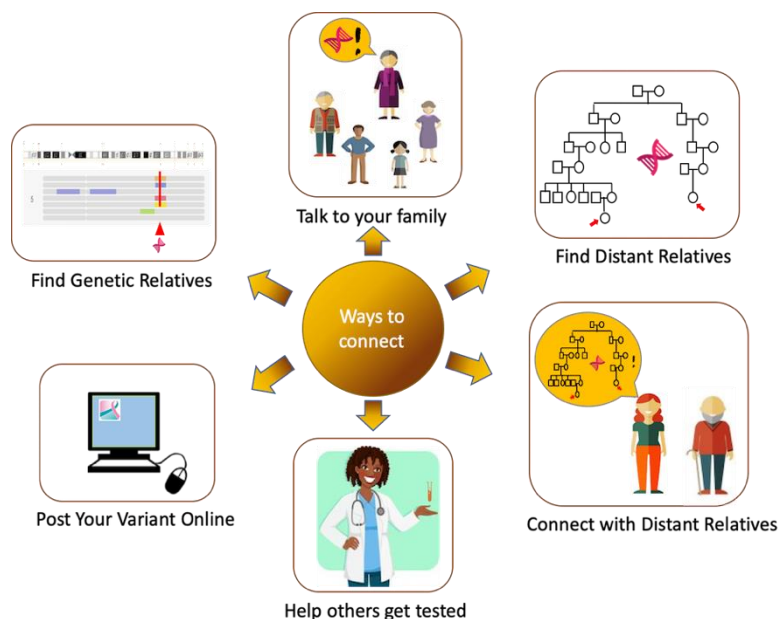
The ConnectMyVariant initiative seeks to help people like you:

- Talk to their relatives
- Share important genetic information
- Expand family trees to identify and connect with distant at-risk relatives
- Guide at-risk relatives to cancer prevention.

“Prevention Through Connection”

People with the same genetic variant may be distantly related through a long-ago ancestor. This means that your family’s variant may be a key to understanding your family’s past. It is also a key that you can use to help both close and distant family members prevent cancer before it happens.

You may have received genetic testing because someone cared enough to warn you about your risk. Now you can find and warn other at-risk relatives. Reaching out and speaking to other at-risk relatives to help them get genetic testing may help prevent cancer and save lives. These are the goals of ConnectMyVariant.



You can learn more and sign up at <http://connectmyvariant.org/>
 Questions? info@connectmyvariant.org