CancerNext-Expanded® +RNAinsight®: Analyses of 77 Genes Associated with Hereditary Cancer

RESULTS

CHEK2  Variant, Unknown Significance:  p.P388S

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- No known clinically actionable alterations were detected.
- No clinically relevant aberrant RNA transcripts were detected in select analyzed genes.*
- 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.P388S (c.1162C>T) 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 (77 total): AIP, ALK, APC*, ATM*, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1*, BRCA2*, BRIP1*, CDC73, CDH1*, CDK4, CDKN1B, CDKN2A, CHEK2*, CTNNA1, Dicer1, FANCC, FH, FLCN, GALNT12, KIF1B, LZTR1, MAX, MEN1, MET, MLH1*, MSH2*, MSH3, MSH6*, MUTYH*, NBN, NF1*, NF2, NTHL1, PALB2*, PHOX2B, PMS2*, POT1, PRKAR1A, PTCH1, PTEN*, RAD51C*, RAD51D*, RB1, RECQL, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARC1, SMARCE1, STK11, SUFU, TMEM127, TP53*, TSC1, TSC2, VHL and XRCC2 (sequencing and deletion/duplication); EGFR, EGLN1, HOXB13, KIT, MITF, PDGFRA, POLD1 and POLE (sequencing only); EPCAM and GREM1 (deletion/duplication only). DNA and RNA analyses performed for * genes.

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-Expanded® +RNAinsight® (Product Code 8874-R)
Methodology: The CancerNext-Expanded® +RNAinsight® test is a comprehensive screen of 77 genes associated with hereditary cancer predisposition. Genomic deoxyribonucleic acid (gDNA) and ribonucleic acid (RNA) are isolated from the patient's specimen using standardized methodology and quantified. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR). 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 DNA analyses include Sanger sequencing 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 thresholds, and potentially homozygous variants are verified by Sanger sequencing. For BRCA2 and MSH2, the Portuguese founder mutation, c.156_157insA (also known as 384insA), and the coding exons 1-7 inversion, respectively, 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 sequenced genes (excluding EGFR, EGLN1, HOXB13, KIT, MITF, PDGFRα, POLD1, POLE, and 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 PMS2CL will be performed to determine if the deletion is located in the PMS2 gene or pseudogene. All sequence analysis is based on the following NCBI reference sequences: AXIN2-NM_004655.3, AIP-NM_00937.2, ALK-NM_004304.4, APC-NM_000398.5 & NM_001127511.2, ATM-NM_000051.3, BAP1-NM_004656.2, BARD1-NM_00465.2, BLM-NM_000057.2, BMP1A-NM_004329.2, BRCA1-NM_007294.3, BRCA2-NM_000059.3, BRIP1-NM_032043.2, CDC73-NM_024529.4, CDH1-NM_004360.3, CDK4-NM_000075.3, CDKN1B-NM_004064.3, CDKN2A-NM_000077.4 and NM_058195.3 (p14ARF), CHEK2-NM_001194.3, CTNNAL1-NM_001903.2, DICER1-NM_177438.2, EGFR-NM_005228.3, EGLN1-NM_002051.2, FANCC-NM_001136.2, FH-NM_001143.3, FLCN-NM_144997.5, GALNT12-NM_024642.4, HOXB13-NM_006361.5, KIF1B-NM_015074.3, KIT-NM_000222.2, LZTR1-NM_000676.3, MAX-NM_000283.2, MEN1-NM_130799.2, MET-NM_000245.1, MITF-NM_000248.3, MÚTYH-NM_001128425.1, MLH1-NM_000249.3, MSH2-NM_000251.1, MSH3-NM_000249.3, MSH6-NM_000179.2, NBN-NM_000285.4, NF1-NM_000267.3, NF2-NM_000268.3, NTHL1-NM_002528.3, PALB2-NM_024675.3, PDGFRα-NM_006206.4, PHOX2B-NM_003924.3, PMS2-NM_000535.5, POLD1-NM_006261.2, POLE-NM_006231.2, POT1-NM_015450.2, PRKAR1A-NM_002794.3, PTC1-NM_000264.3, PTEN-NM_000314.4, RAD51C-NM_058216.1, RAD51D-NM_002878.3, RBL1-NM_000321.2, RECO1L-NM_002907.3, RET-NM_002975.4, SDHA-NM_004168.2, SDHAF2-NM_017841.2, SDHB-NM_000300.2, SDHC-NM_000301.2, SDHD-NM_000302.2, SMAD4-NM_005359.5, SMARCA4-NM_001128489.1, SMARCA1-NM_003073.3, SMARCA1-NM_002079.4, STRK11-NM_000455.4, SUFU-NM_016169.3, TME127-NM_017849.3, TP53-NM_000254.6, TSC1-NM_000368.4, TSC2-NM_000458.4, VHL-NM_000551.3, XRC2-NM_005431.1.

Analytical Range: The CancerNext-Expanded® +RNAinsight® test targets detection of DNA sequence mutations in 75 genes 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 untranslatable regions. For HOXB13, only variants impacting codon 84 are routinely reported. For MITF, only the status of the c.952G>A (p.E318K) alteration is analyzed and reported. For EGFR, only the status of the c.2369C>T (p.T790M) and c.2327G>A (p.R776H) alterations are analyzed and reported. For EGLN1, only missense variants in the catalytic domain (codons 188-418) are reported. For RECO1L, only missense variants in the helicase and RCQ domains (codons 63-592) and exonic truncating variants 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 ALK, only variants located within the kinase domain (c.3286-c.4149) are reported. For PDGFRα, only missense variants or in-frame insertion/deletions located in coding exons 10, 12, 14, and 18 are reported. For KIT, only missense variants or in-frame insertion/deletions located in coding exons 8, 9, 11, 13, and 17 are reported. The MSH3 exon 1 repeat region and the PHOX2B polyalanine repeat region are excluded from analysis. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions for the sequenced genes (excluding EGFR, EGLN1, HOXB13, KIT, MITF, PDGFRα, POLD1, POLE) plus EPCAM and GREM1. 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 promotor 1B gross deletions as well as single nucleotide substitutions within the promotor 1B YY1 binding motif (NM_001127511.1-c.-196_-186) are analyzed and reported. RNA transcripts are screened for 18 genes (APC, ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, NF1, PALB2, PMS2 exons 1-10, PTEN, RAD51C, RAD51D, and TP53) and compared to a human reference pool. The absence or presence of RNA transcripts meeting quality thresholds are incorporated as evidence towards assessment and classification of DNA variants. Any regions not meeting RNA quality thresholds are excluded from analysis. Regions routinely excluded due to chronically low expression in human peripheral lymphocytes include: BRCA2 (exon 1), BRIP1 (exons 18, 20), CDH1 (Exons 1, 2, 16), and CHEK2 (exons 1, 7, 8).

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

- **Pathogenic Mutation**: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Previously described pathogenic mutations, including intrinsic mutations at any position, are always reported when detected.

- **Variant, Likely Pathogenic (VLP)**: alterations with strong evidence in favor of pathogenicity. Previously described likely pathogenic variants, including intrinsic VLPs at any position, are always reported when detected.

- **Variant, Unknown Significance (VUS)**: alterations with limited and/or conflicting evidence regarding pathogenicity. Intrinsic 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.

7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.

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 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, 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 77 genes represented on the test (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Cancer is a complex clinical disorder. 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.
CHEK2 NM_007194 c.1162C>T p.P388S

VARIANT DETAILS:

The p.P388S variant (also known as c.1162C>T), located in coding exon 10 of the CHEK2 gene, results from a C to T substitution at nucleotide position 1162. The proline at codon 388 is replaced by serine, an amino acid with similar properties. This amino acid position is highly conserved in available vertebrate species. In addition, the in silico prediction for this alteration is inconclusive. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear.

GENE INFORMATION:


ADDITIONAL SUPPORTING INFORMATION:

<table>
<thead>
<tr>
<th>Co-Segregation</th>
<th>Co-segregation data for this variant is currently unavailable.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-occurrence</td>
<td>No significant co-occurrence data is currently available at our laboratory.</td>
</tr>
<tr>
<td>Frequency</td>
<td>Internal Frequency: This alteration has not been previously detected at our laboratory (398000 total alleles studied). No population frequency information could be found.</td>
</tr>
<tr>
<td>Grantham Score</td>
<td>74 (similar amino acid substitution)</td>
</tr>
<tr>
<td>in silico</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>
Evolutionary conservation diagram: Amino Acid Alignment

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

Amino Acid Change:

<table>
<thead>
<tr>
<th>Trait</th>
<th>Pro (P)</th>
<th>Ser (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid Name</td>
<td>Proline</td>
<td>Serine</td>
</tr>
<tr>
<td>Polarity/Charge</td>
<td>non-polar</td>
<td>polar</td>
</tr>
<tr>
<td>pH</td>
<td>neutral</td>
<td>neutral</td>
</tr>
<tr>
<td>Residue Weight</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>Hydrophobicity Score</td>
<td>-1.6</td>
<td>-0.8</td>
</tr>
<tr>
<td>Hydropilicity Score</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Secondary Structure Propensity</td>
<td>strong α breaker / strong β breaker</td>
<td>α indifferent / β breaker</td>
</tr>
</tbody>
</table>

Grantham Difference: 74.00
# Understanding Your VUS Hereditary Cancer Genetic Test Result

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

<table>
<thead>
<tr>
<th>Result</th>
<th>VUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reclassification</td>
<td>POSSIBLE</td>
</tr>
<tr>
<td>Cancer Risk</td>
<td>VARIES</td>
</tr>
<tr>
<td>Risk Management</td>
<td>VARIES</td>
</tr>
<tr>
<td>Family Members</td>
<td>POSSIBLE FURTHER TESTING</td>
</tr>
<tr>
<td>Next Steps</td>
<td>DISCUSS</td>
</tr>
<tr>
<td>Reach Out</td>
<td>RESOURCES</td>
</tr>
</tbody>
</table>

- 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.

- 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.

- 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 decisions are very personal and depend on many factors. Talk to your doctor about which, if any, options may be right for you.

- 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.

- It is recommended that you stay in contact with your healthcare provider on a regular basis for possible new information about your result.

- **Ambry’s Hereditary Cancer Site for Families** patients.ambyrgen.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.
Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A VARIANT OF UNKNOWN SIGNIFICANCE

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

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.
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.