CYGENEX
A precision medicine solutions company

Patient Information: Accession: Physician: Report Date:

Final Report

## TEST PERFORMED

**CGX Comprehensive Cancer**
(44 Gene Panel; gene sequencing with deletion and duplication analysis)

## RESULTS:

No clinically significant sequence or copy-number variants were identified which are sufficient for a molecular diagnosis.

However, one variant of potential clinical relevance is reported.

### *Actionable Variants*

*None*

### *Additional Variants*

| Gene Info | | Variant Info | | |
|---|---|---|---|---|
| GENE | INHERITANCE | VARIANT | ZYGOSITY | CLASSIFICATION |
| *MUTYH* NM_001128425.1 | Autosomal Recessive | c.1187G>A p.Gly396Asp | Heterozygous | **Pathogenic** (carrier)** |

**** Heterozygous carriers of the above reported pathogenic MUTYH variant may be at increased risk for associated cancers. However, this has not yet been conclusively demonstrated.**

## CLINICAL INTERPRETATIONS AND RECOMMENDATIONS:

**Other Variants, Notes, and Recommendations:**

- As requested, this report only includes variants classified as Pathogenic, Likely Pathogenic, or Risk Allele at the time of analysis. If detected, this report does not include variants classified as of uncertain significance.
- Gene specific limitations may be present, see "Test Summary".
- These results should be interpreted in the context of this individual's clinical findings, biochemical profile, and family history.
- Genetic counseling is recommended. Available genetic counselors and additional resources can be found at the National Society of Genetic Counselors (NSGC)

## TEST SUMMARY:

44 genes tested (99.99% at >50x).

*APC, ATM, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FANCA, FANCB, FANCC, FANCD2, FANCE, FH, MEN1, MET, MITF, MLH1, MRE11, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RET, SMAD4, STK11, TP53, VHL*

**Gene Specific Notes and Limitations**

- Annotation and analysis of the *CDKN2A* gene includes the sequence for two protein products of this gene, p16 (NM_000077.4) and p14ARF (NM_058195.3).
- *EPCAM* gene testing is limited to deletion and duplication analysis (PubMed: 23264089).
- Due to pseudogene interference, copy-number-variants within exon 14-17 of the *FANCD2* gene (NM _033084.4) are not evaluated and

detection of single-nucleotide variants and small insertions/deletions in this region is not guaranteed.

- Inversion of *MSH2* exons 1-7 ("Boland" inversion) is assessed for Lynch Syndrome, Colorectal, and Endometrial Cancer Panel testing (for both Focus and Comprehensive Panels) as well as Comprehensive Gastric Cancer Panel testing. Unless otherwise specified, this testing is not performed for other cancer panels, but is available upon request.
- The ordered testing method does not guarantee the detection of potentially causative somatic changes in *NF1*.
- The whole *PMS2* gene is assessed for both sequence and copy-number variants (if del/dup is ordered). Variants located in the region homologous to the pseudogene *PMS2CL* (exons 12-15) will be confirmed by long range PCR. Exception: a specific variant, NM_000535.6:c.2186_2187del (p.Leu729Glnfs*6), may not be detected due to pseudogene interference unless there is clinical suspicion for Lynch Syndrome. Potential copy number variants located in the region homologous to the pseudogene *PMS2CL* will be tested by LR-PCR with NGS. The sensitivity of this approach may not be as high as for copy number variants in other genes.
- Evidence for LOF variants in the *POLD1* gene to be involved in cancer predisposition is limited and unlikely based on the expected mechanisms (PubMed: 23263490, 23447401). Thus such variants, including copy-number variants, are typically not reported on Hereditary Cancer Panels if detected.
- Loss of function variants in the *POLE* gene are associated with autosomal recessive IMAGEI syndrome (OMIM: 618336). Evidence for LOF variants to be involved in cancer predisposition is limited and unlikely based on the expected mechanisms (PubMed: 23263490, 23447401). Thus such variants, including copy-number variants, are typically not reported on Hereditary Cancer Panels if detected.
- The *PTEN* promoter region is analyzed for both sequencing and copy number variants.
- Copy-number analysis of the *RAD50* gene is limited to variants encompassing at minimum two consecutive exons.

INTERPRETATION:

## About *MUTYH*

Biallelic germline mutations in *MUTYH* can result in MUTYH-associated polyposis (MAP), a colorectal cancer predisposition syndrome inherited in an autosomal recessive manner, which is characterized by the development of large numbers of colon polyps and severely increased risk for colon cancer, although some cases have been reported with colorectal cancer development in absence of polyps. Additional population studies also showed an increased risk for heterozygous carriers, although this association becomes almost insignificant in meta analyses. However, in family based studies, the associated risk was found to be much more significant than in general population based studies, indicating that heterozygous carriers may be at increased risk, particularly with positive a family history of colorectal cancer. Further studies may be necessary to clarify these findings (PubMed: 23035301; OMIM: 604933).

The gene product of the *MUTYH* gene is a protein called MutY DNA glycosylase. See OMIM gene entry for *MUTYH* (OMIM: 604933) for further information.

100% of the coding sequence of the NM_001128425.1 transcript of *MUTYH* gene was sequenced to a minimum depth of 50x in the submitted specimen. Neither a second sequencing mutation nor a copy number variant was detected in this gene; however, the presence of mutations in the deep intronic or regulatory regions cannot be ruled out. As the clinical condition(s) associated with mutations in the *MUTYH* gene are recessive and only a single heterozygous variant has been detected, this result is interpreted as carrier status only. Further clinical evaluation may be warranted to clarify these findings.

### *MUTYH* NM_001128425.1:c.1187G>A (p.Gly396Asp)

Classification: **Pathogenic**

**Zygosity and Inheritance**

- This heterozygous Pathogenic variant is consistent with this individual being a carrier for an autosomal recessive *MUTYH*-related condition.

**Variant Type**

- Genomic change: Chr1(GRCh37):g.45797228C>T.
- This variant is in the dbSNP database: rs36053993
- This variant is predicted to result in a single amino acid substitution (missense) of **Gly** to **Asp** at codon 396 in exon 13 of the *MUTYH* gene.
- This variant is located near an intron-exon boundary.

Variant in Cases

- This variant, p.Gly396Asp, also known as p.Gly382Asp, has been reported as a founder mutation in Europeans in the homozygous and compound heterozygous states in affected individuals with MUTYH-associated polyposis, colorectal cancer, breast and endometrial cancer, pancreatic adenocarcinoma, and other conditions (PubMed: 19032956, 17489848, 19793053, 12606733, 30067863) and has been found to co-segregate with disease in multiple families (PubMed: 11818965, 16557584, 17489848).
- This variant is classified as "Pathogenic" or "Likely Pathogenic" in ClinVar, with multiple submitters in agreement (ClinVar: 5294).
- This variant is classified as a "Disease Mutation" (DM) in the Human Gene Mutation Database (HGMD).
- This variant has one or more entries in ClinVar: RCV000515320.1, RCV000586537.2, RCV000079501.11, RCV000501239.1, RCV000115748.17, RCV000121598.2, RCV000144637.1, RCV000493920.1, RCV000477907.1, RCV000005615.4, RCV000005614.24

Variant in Controls

- This variant has been observed at a frequency of 0.31% (860/281146 alleles).
- The highest allele frequency that this variant has been observed at in any sub-population with available data is 0.49% in the European (Non-Finnish) population.
- There are three homozygous control individuals for this variant.
- The Broad Institute gnomAD database (>120,000 Individuals with no known severe, pediatric onset disease) was used for this analysis.

Other Variant Information

- *In vitro* studies suggest that this variant will impair the function of the protein product encoded by this gene (PubMed: 18534194, 15987719, 19836313).
- Analysis of amino acid conservation indicates that the wild-type amino acid Gly is completely conserved across vertebrate species, suggesting that a change at this position may not be tolerated and could adversely affect the structure and/or function of the protein.
- Amino acid conservation data:
  - Primates: 12 out of 12 match the wild type.
  - Mammals: 62 out of 62 match the wild type.
  - Vertebrates: 93 out of 93 match the wild type.
- The physiochemical difference between Gly and Asp as measured by Grantham's Distance is 94. This score is considered a "moderate" change. (PubMed: 4843792, 6442359).
- Computational predictions for p.Gly396Asp (2P/0B AGVGD, SIFT/) (REVEL = 0.551) (gnomAD: Z = -0.20721 [Exp: 311.71, Obs: 322]) (granthamDist = 94).

## METHODS:

Genomic DNA from the submitted sample is barcoded and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries are sequenced using a Next Generation Sequencing (NGS) technology. Sequencing results are aligned to the human genome reference sequence (assembly GRCh37) and variants are detected in regions with sufficient coverage. 99.99% of coding regions and splicing junctions are covered to at least 50x by NGS or by Sanger sequencing. Locus specific databases, literature searches, and other molecular biological principles are used to classify variants. To minimize false positive results, any variants that do not meet internal quality standards are confirmed by Sanger sequencing. Variants classified as pathogenic, likely pathogenic, or risk allele which are located in the coding regions and nearby intronic regions (+/- 20bp) of the genes listed above are reported. Variants outside these intervals may be reported but are typically not guaranteed. When a single pathogenic or likely pathogenic variant is identified in a clinically relevant gene with autosomal recessive inheritance, the laboratory will attempt to ensure 100% coverage of coding sequences either through NGS or Sanger sequencing technologies ("fill-in"). All genes listed were evaluated for large deletions and/or duplications. Deletions or duplications identified by NGS are confirmed by an orthogonal method (qPCR or MLPA), unless exceeding an internally specified and validated quality score, beyond which deletions and duplications are considered real without further confirmation. New York patients: diagnostic findings are confirmed by Sanger, MLPA, or qPCR; exception SNV variants in genes for which confirmation of NGS results has been performed >=10 times may not be confirmed if identified with high quality by NGS. Bioinformatics: The Fulgent Germline v2019.2 pipeline was used to analyze this specimen.

## LIMITATIONS:

Test results and variant interpretation are based on the proper identification of the submitted specimen and use of correct human reference sequences at the queried loci. In very rare instances, errors may result due to mix-up or co-mingling of specimens. Positive results do not imply that there are no other contributions, genetic or otherwise, to the patient's phenotype, and negative results do not rule out a genetic cause for the indication for testing. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (https://www.genenames.org) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation is based on the collected information and Alamut annotation available at the time of reporting. This assay is designed and validated for detection of germline variants only. This assay is not designed or validated for the detection of mosaicism, including changes commonly reported in association with blood malignancies. This test is not designed or validated to detect the presence and/or break points of copy-number-neutral gross-chromosomal rearrangements such as translocations. DNA alterations in regulatory regions or deep intronic regions (greater than 20bp from an exon) will not be detected by this test. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical, computational, or human error, DNA variants may be missed. Although next generation sequencing technologies and our bioinformatics analysis significantly reduce the confounding contribution of pseudogene sequences or other highly-homologous sequences, sometimes these may still interfere with the technical ability of the assay to identify pathogenic variant alleles in both sequencing and deletion/duplication analyses. Deletion/duplication analysis can identify alterations in genomic regions and is evaluated at a single exon resolution level in relevant genes associated with the patient's clinical presentation. For custom added genes and applicable genes that may be of interest, deletion/duplication analysis is evaluated at a resolution of two or more contiguous exons. When novel DNA duplications are identified, it is not possible to discern the genomic location or orientation of the duplicated segment, hence the effect of the duplication cannot be predicted. Where deletions are detected, it is not always possible to determine whether the predicted product will remain in-frame or not. Unless otherwise indicated, in regions that have been sequenced by Sanger, deletion/duplication analysis has not been performed.

## SIGNATURE:

**Dr. Harry Gao, DABMG, FACMG** on **7/8/2021 09:50 AM PDT**
Electronically signed

## DISCLAIMER:

This test was developed and its performance characteristics determined by Fulgent Genetics CAP #8042697 CLIA #05D2043189; 4978 Santa Anita Ave., Suite 205, Temple City, CA 91780. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. For assistance with interpretation of these results, healthcare professionals may contact us directly at **800-516-1676**. It is recommended that patients receive appropriate genetic counseling to explain the implications of the test result, including its residual risks, uncertainties and reproductive or medical options.