Golden Labs Tests

Genomics Testing Lab
Entprise Blvd, Bozeman MT
Phone: (406) 555-6666 / Fax: (406) 555-7777
http://goldenlabs.org/tests

Name: **RD-BATCH5-SAMPLE27**

Accession ID: **A1234**

DOB: **1541606599448**
Sex: **Female**
Race/Ethnicity: **Other**
Family #: **F123**

MRN: **MRN12345**
Referring facility: **Dr. McCoy**
Referring physician: **Bones**
Copies to: **USS Enterprise**

Specimen: **Blood**
Date of Collection: **10/10/2018**
Date of Receipt: **10/24/2018**
Date of Report: **11/7/2018**

Test(s) Performed: **Tageted gene panel sequencing**
Indication for test: **Familiar Risk of Breast Cancer**

RESULT: Positive
Findings explain patient phenotype, Incidental findings identified.

APPROACH

Sequencing of select genes was done using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. A total of N genes with previous implications in various mendelian disorders (see Supplement for a list of genes and coverage information) were covered with minimum read depth of 30X. Note that this test cannot exclude the possibility of variants in genes not analyzed or assayed with incomplete coverage.

VARIANTS RELEVANT TO INDICATION FOR TESTING

One pathogenic variant in RAF1 was identified in this individual. No other variants of relevance to the indication were identified. Please see below for more detailed variant information.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
RAF1 NM_002880.3	c.770C>T p.Ser257Leu	Het.	Exon 7	Noonan Syndrome 5	Autosomal Dominant / Heterozygous	Pathogenic
BRCA2 NM_000059.3	Copy Number Gain	Dup.	Exons 17-20	Hereditary Breast Cancer	Dominant	Likely Pathogenic

OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

Incidental findings are variants of medical significance that are not associated with the individual's reported indication. Please note that the presence of pathogenic variants in genes with incomplete coverage or in genes not examined cannot be fully excluded.

Monogenic Disease Risk

This individual has one variant in a gene associated with a dominant disorder that is unrelated to this individual's reported phenotype. In the heterozygous state, this variant may infer a partial or complete risk of acquiring the disease if it has not manifested. Please see below for more detailed variant information.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
EGFR NM_005228.4	c.2284-5_2290dupTCCAGGAAGCCT p.Ala763_Tyr764insPheGlnGluAla	Het.	Exon 20	Lung Cancer	Autosomal Dominant / Heterozygous	Likely Pathogenic

Carrier Status

This individual is a carrier of one heterozygous pathogenic variant in a gene associated with a recessive disorder that is unrelated to this individual's reported phenotype. In the heterozygous state, this variant is not known to play a role in disease. Please see below for more detailed variant information.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
MLH1 NM_000249.3	c.2103G>A p.Gln701=	Het.	Exon 18	Colorectal Cancer, Hereditary Nonpolyposis, Type 2	Autosomal Recessive / Heterozygous	Likely Pathogenic

RECOMMENDATIONS

The interpretation of these results should be done in the context of a patient's medical record and family history. Please note that interpretation and classification of the variants reported here may change over time. Please see a genetic counselor for services regarding the implications of these results in the context of understanding the implications of incidental findings, family planning and the informing of family members of potentially shared genetic outcomes.

DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)

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Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
RAF1 NM_002880.3	c.770C>T p.Ser257Leu	Het.	Exon 7	Noonan Syndrome 5	Autosomal Dominant / Heterozygous	Pathogenic
Genomic Position			**Variant Frequency**			
Chr3:NC_000003.11:g.12645699G>A			Not identified in large population studies			

VARIANT INTERPRETATION: The missense variant p.S257L in RAF1 (NM_002880.3) causes the same amino acid change as a previously established pathogenic variant. The p.S257L variant is novel (not in any individuals) in gnomAD Exomes and is novel (not in any individuals) in 1000 Genomes.There is a large physicochemical difference between serine and leucine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. The gene RAF1 has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 2.82. The gene RAF1 contains 34 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. 19 variants within 6 amino acid positions of the variant p.S257L have been shown to be pathogenic, while none have been shown to be benign. The p.S257L missense variant is predicted to be damaging by both SIFT and PolyPhen2. The serine residue at codon 257 of RAF1 is conserved in all mammalian species. The nucleotide c.770 in RAF1 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Pathogenic.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
BRCA2 NM_000059.3	Copy Number Gain	Dup.	Exons 17-20	Hereditary Breast Cancer	Dominant	Likely Pathogenic
Genomic Position			**Ratio / Z-Score compared to Reference Samples, Mean Read Depth**			
Chr13:32936640-32945257			1.404 Ratio, 3.785 Z-Score, 806.244 Mean Read Depth			

VARIANT INTERPRETATION: This variant is a copy number gain of the genomic region encompassing exons 14-24 of the BRCA2 gene. Four copies of this region have been detected, but the exact position of these copies in the genome is not known. However, sub-genic duplications are generally in tandem (PMID: 25640679) and it is likely that three copies of this region (i.e. a triplication) are in tandem and may result in an absent or disrupted protein product This variant has not been reported in the literature in individuals with BRCA2-related disease. Experimental studies and prediction algorithms are not available for this variant, and the functional significance of the duplicated amino acids is currently unknown. Loss-of-function variants in BRCA2 are known to be pathogenic (PMID: 20104584). In summary, the currently available evidence indicates that the variant is pathogenic, but additional data are needed to prove that conclusively. Therefore, this variant has been classified as Likely Pathogenic.

DETAILED VARIANT INFORMATION (INCIDENTAL FINDINGS)

Monogenic Disease Risk

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
EGFR NM_005228.4	c.2284-5_2290dupTCCAGGAAGCCT p.Ala763_Tyr764insPheGlnGluAla	Het.	Exon 20	Lung Cancer	Autosomal Dominant / Heterozygous	Likely Pathogenic
Genomic Position			**Variant Frequency**			
Chr7:NC_000007.13:g.55248992_55248993insTCCAGGAAGCCT			Not identified in large population studies			

VARIANT INTERPRETATION: The in-frame insertion p.A763_Y764insFQEA in EGFR (NM_005228.4) has been reported to ClinVar as Likely Pathogenic with a status of (1 stars) criteria provided, single submitter (Variation ID 45248 as of 2018-09-04).. The p.A763_Y764insFQEA variant is novel (not in any individuals) in gnomAD Exomes and is novel (not in any individuals) in 1000 Genomes.This variant results in a insertion of 4 amino acid residues starting at 764, including PheGlnGluAla. However, as this is an in-frame insertion, it is not expected to result in either a truncated protein product or loss of protein through nonsense-mediated mRNA decay. The p.A763_Y764insFQEA variant is not in a repeat region. The p.A763_Y764insFQEA variant results in a insertion of a base that is predicted conserved by GERP++ and PhyloP. 2 variants within 6 amino acid positions of the variant p.A763_Y764insFQEA have been shown to be pathogenic, while none have been shown to be benign. The nucleotide c.2291 in EGFR is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Likely Pathogenic.

Carrier Status

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
MLH1 NM_000249.3	c.2103G>A p.Gln701=	Het.	Exon 18	Colorectal Cancer, Hereditary Nonpolyposis, Type 2	Autosomal Recessive / Heterozygous	Likely Pathogenic
Genomic Position			**Variant Frequency**			
Chr3:NC_000003.11:g.37090508G>A			Not identified in large population studies			

VARIANT INTERPRETATION: The synonymous variant p.Q701= in MLH1 (NM_000249.3) has been reported to ClinVar as Likely Pathogenic with a status of (3 stars) reviewed by expert panel (Variation ID 90048 as of 2018-09-04).. The p.Q701= variant is novel (not in any individuals) in gnomAD Exomes and is novel (not in any individuals) in 1000 Genomes. The p.Q701= variant is predicted to disrupt splicing by all splice site algorithms. The nucleotide c.2103 in MLH1 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Likely Pathogenic.

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METHODOLOGY

The individual's DNA was extracted and fragmented, with fragments from the coding regions of the select gene panel targeted for amplification and sequencing. Reads from the sequence output were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner (BWA). Variants to the reference were called using the Genomic Analysis Tool Kit (GATK). The variants were annotated and filtered using the Golden Helix VarSeq analysis workflow implementing the ACMG guidelines for interpretation of sequence variants. This includes comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes Project Consortium's publication of 2,500 genomes, the NCBI ClinVar database of clinical assertions on variant's pathogenicity and multiple lines of computational evidence on conservation and functional impact. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

VARIANT ASSESSMENT PROCESS

The following databases and in-silico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCIB RefSeq Genes, ExAC Gene Constraints, VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using the to HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar.

LIMITATIONS

It should be noted that this test is limited to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meets a level of evidence threshold for cause or contribute to disease. Certain classes of genomic variants are also not covered using the NGS testing technology, including triplet repeat expansions, copy number alterations, translocations and gene fusions or other complex structural rearrangements. More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.

REFERENCES

Richards, Sue, et al. "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology." Genetics in medicine 17.5 (2015): 405.
Exome Aggregation Consortium et al. "Analysis of Protein-Coding Genetic Variation in 60,706 Humans." Nature 536.7616 (2016): 285–291. PMC. Web. 13 May 2018.
The 1000 Genomes Project Consortium. "A Global Reference for Human Genetic Variation." Nature 526.7571 (2015): 68–74. PMC. Web. 13 May 2018.

DRAFT REPORT