otogenetics® corporation

Patient: #####12345-COt####
CLIA ID#: 11D2066426
First Last, MD, Lab Director

Hereditary Cancer Risk Assessment Testing Report

Patient Information

Patient Name: Last, First
Date of Birth: Mot ##, yyyy
Age: ##
Sex: xxxxxxxxxxx
Ethnicity: xxxxxxxxxxxxxxx

Provider Information

Provider: ##### Clinics
Provider ID: ##########
Physician: Last, First

Specimen

Accession ID: #####12345
Sample ID: COt####
Specimen Type: Buccal Swab
Collection Date: Aug ## 2017
Report Date: Sep #, 2017

Patient Results: Positive - Pathogenic Variant(s) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
FANCC NM_000136.2: c.355_358delTCTC p.S119fs*24	Deletion	Heterozygous	rs750003253	hereditary cancer	Pathogenic

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
FANCC	5	c.355_358delTCTC	p.S119fs*24	rs750003253	Het	Pathogenic

FANCC is a DNA repair protein involved in genome maintenance by regulating the DNA damage control pathway. The FANCC protein is one of a group of proteins known as the FA core complex. The FA core complex is composed of eight FA proteins (including FANCC) and two proteins called Fanconi anemia-associated proteins (FAAPs). This complex activates two proteins, called FANCD2 and FANCI, by attaching a single molecule called ubiquitin to each of them (a process called monoubiquitination). The activation of these two proteins, which attach (bind) together to form the ID protein complex, attract DNA repair proteins to the area of DNA damage so the error can be corrected and DNA replication can continue. Mutations in the FANCC gene can lead to absent or reduced protein function. As a result, the FA complex cannot function and the entire FA pathway is disrupted. Due to the disrupted pathway, DNA damage is not repaired efficiently. When the buildup of errors in DNA leads to uncontrolled cell growth, affected individuals can develop leukemia or other cancers. Somatic alterations have been reported in 2.5% of colon cancers, 1.4% of melanomas, and 1.1% of NSCLCs.

Additional Comments

A variant known to be associated with increased risk in hereditary Cancers is detected.
Please note:
1. This report is based on the selected genes included in the Hereditary Cancer Risk Assessment Test, and does not predict risks associated with other genes or unknown genes not included in the test.
2. Only the protein coding regions and splicing sites of the targeted genes are included in this analysis and reported herein. Sequences outside the sequenced regions of these genes are not sequenced for genetic risk analysis.

Otogenetics Corporation | 4553 Winters Chapel Road, Suite 100, Atlanta, GA 30360 | www.otogenetics.com | Customer support: support@otogenetics.com
QIAGEN Clinical Insight - Interpret software was used in sequence interpretation.

3. Certain types of mutations other than single nucleotide changes or smaller deletion/duplication may not be identified based on current Next Generation Sequencing analysis technology used in this test.
4. Future findings may provide new clinical interpretation of certain variants.

Followup Recommendations

Follow up with your healthcare providers for clinical correlation with personal and family medical history, testing of family members, and updated genetic risk information.

Genes Tested

Targeted regions for “Inherited Cancer Gene Panel” include the exonic regions of the following genes: APC; ATM; BARD1; BMPR1A; BRCA1; BRCA2; BRIP1; CDH1; CDK4; CDKN2A; CHEK2; ELAC2; EPCAM; FANCC; HRAS; MEN1; MET; MLH1; MRE11A; MSH2; MSH6; MUTYH; NBN; NF1; NTRK1; PALB2; PALLD; PMS2; PTCH1; PTEN; RAD50; RAD51; RAD51C; RAD51D; RET; SMAD4; STK11; TP53; VHL.

Methods and Limitations

Sample Processing and Sequencing and Variant Detection - Developed by Otogenetics (2015), this gene panel focuses on the coding exonic regions of genes annotated in HG19 reference genome. Genomic targets were identified based on information in the Human Gene Mutation Database (HGMD), the Online Mendelian Inheritance in Man (OMIM) catalog, GeneTests.org, Illumina TruSight sequencing panels, and other commercially available sequencing panels. Combining data from these sources ensured that genes currently identified in clinical research settings as pathogenic were included in the panel. Standard Operation Procedures were used to process the samples. Genomic DNA was extracted from clinical samples (saliva, blood, swab, or as specified in the report), library preparation via Illumina protocols, capture based enrichment of a targeted region was performed by solution-based hybridization which enriches for coding regions of targeted genes with specific probes. Multiple quality control steps were performed for sample and derivative quality evaluation. Sequencing was performed using Illumina Sequencer(s), and a minimum average coverage depth of 100X was required.

Variant call Format (VCF) Generation - VCF file was generated using either the Sentieon analysis pipeline or the Best Practices from GATK pipeline on DNAnexus platform. Reference genome used is UCSC hg19. Additional quality filters, including quality score of 20 and a minimum coverage (DP) of 8x, were applied to generate the VCF subjected to QIAGEN Clinical Insight interpretation for reporting as described below.

Limitations - Absence of a primary diagnostic finding identified by this test does not exclude the possibility of a genetic basis for the clinical condition for this proband. Variants in the intronic, UTR and promoter regions and other copy number variants are not intended to be detected by this assay.

Specifically, detection of abnormal variants depends on the presence of these sequence variants in the targeted region that was sequenced. It is possible that the gene region where a disease causing mutation exists in the patient was not captured using the current technologies of this test and therefore was not detected.

This sequence test is designed to evaluate single nucleotide variants, 1-3 nucleotide variants and small insertions and deletions (<10 nucleotides) within the targeted region. CNV kit analysis was applied to assess large deletions and duplications. The current technology targets the coding exonic regions of the 39 genes and not the 5’ or 3’ untranslated regions, promoter or splice sites of these genes. Thus, a variant in these non-coding exonic regions will not be sequenced at high depth, and may not be identified in this test. Coverage within the target region may also influence the identification of variants.

Additionally, certain types of genetic abnormalities are difficult to identify in sequencing data and have not been validated for clinical use including but not limited to insertions, deletions, copy number alterations, long repetitive sequences, triplet repeat expansions, chromosomal rearrangements, polyploidy, repetitive regions including mono-, di- and tri-nucleotide repeats, GX rich regions, intronic variants outside the splice-site and epigenetic effects.

Hereditary Cancer Risk Assessment Testing Report

It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes. Clinical correlation and periodic review of scientific and medical literature is recommended to determine whether Variants of Unknown Significance may be consistent with the patient's phenotype.

Validation is recommended for exons with depth of coverage <8x. We recommend that variants of interest which do not meet the coverage minimum be confirmed clinically before treatment is undertaken. Validation of variants reported is also recommended.

Access to sequencing data, intermediate data files, and detailed analysis tools applied is available upon request.

QIAGEN Clinical Insight - Interpret software was used in sequence analysis and interpretation. The application was internally designed and developed by QIAGEN. All analyses were based on: QIAGEN Clinical Insight-Interpret (5.0.20170822), Ingenuity Knowledge Base (Narnia 170825.001), CADD (v1.3), CentoMD (-), EVS (ESP6500SI-V2), Allele Frequency Community (2017-07-03), JASPAR (2013-11), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), gnomAD (2.0.1), Clinical Trials (Narnia 170825.001), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2017-06-01), DGV (2016-05-15), COSMIC (v81), ExAC (0.3.1), HGMD (2017.2), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150), TargetScan (6.2), SIFT4G (2016-02-23). Weekly updates to Ingenuity Knowledge Base for clinical trials recruitment status and new findings from recent articles. Variants are reported according to HGVS nomenclature and were classified following ACMG guidelines. Information on therapeutic agents and clinical trials were obtained from publicly available information. Variants, therapies, and trials listed in this report are not ranked in order of potential clinical significance or predicted efficacy for this patient.

Laboratory Statement

This Laboratory Developed Test for Next-Generation sequencing of genomic DNA was developed and its performance characteristics established by Otogenetics Corporation, Atlanta, GA. This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing and has validated the test's accuracy according to CAP proficiency testing. This test has not been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test was performed by Otogenetics, a CLIA-certified laboratory (CLIA ID#: 11D2066426)

Selected Citations

Esteban-Jurado C, Franch-Expósito S, Muñoz J, Ocaña T, Carballal S, López-Cerón M, Cuatrecasas M, Vila-Casadesús M, Lozano JJ, Serra E, Beltran S, Brea-Fernández A, Ruiz-Ponte C, Castells A, Bujanda L, Garre P, Caldés T, Cubiella J, Balaguer F, Castellví-Bel S (2016) The Fanconi anemia DNA damage repair pathway in the spotlight for germline predisposition to colorectal cancer. Eur J Hum Genet 2016 Oct;24(10):1501-5 https://www.ncbi.nlm.nih.gov/pubmed/27165003?dopt=Abstract PubMed PMID: 27165003