Hereditary Cancer Risk Assessment Testing Report

Patient Information	**Provider Information**	**Specimen**
Patient Name xxxxx, yyyy	Provider XXXX Clinics	Accession ID #####121345
Date of Birth Mot dd, yyyy	Provider ID ###########	Sample ID COt####
Age ##		Specimen Type Saliva (or swab, or blood)
Sex xx	Physician Last, First	Collection Date Mot 8, 2017
Ethnicity xxxxxx		Report Date Mot 17, 2017

Patient Results: Positive - Pathogenic Variant(s) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
BRCA2 NM_000059.3: c.778_779delGA p.E260fs*15	Deletion	Heterozygous	rs80359677; rs80359680	hereditary cancer	Pathogenic

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
BRCA2	9	c.778_779delGA	p.E260fs*15	rs80359677; rs80359680	Het	Pathogenic

BRCA2 is a tumor suppressor that is involved in genetic stability through aberrant DNA damage repair [PMID:15829967]. Deletions, loss of heterozygosity, and loss-of-function mutations cause BRCA2 inactivation [PMID:8524414, 16551709]. A similar 2 nucleotide deletion mutation of BRCA2 gene (deletion c.775_776delAG [germline]) is observed with breast cancer in human (Canadian). [PMID:8673090]

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.
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

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Followup Recommendations

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

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.

It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to

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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 (5.0.20170801), Ingenuity Knowledge Base (Narnia 170813.001), 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 170813.001), 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

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005 Apr 14;434(7035):917-21 https://www.ncbi.nlm.nih.gov/pubmed/15829967?dopt=Abstract PubMed PMID: 15829967

Phelan CM, Lancaster JM, Tonin P, Gumbs C, Cochran C, Carter R, Ghadirian P, Perret C, Moslehi R, Dion F, Faucher MC, Dole K, Karimi S, Foulkes W, Lounis H, Warner E, Goss P, Anderson D, Larsson C, Narod SA, Futreal PA (1996) Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. Nat Genet. 1996 May;13(1):120-2 https://www.ncbi.nlm.nih.gov/pubmed/8673090?dopt=Abstract PubMed PMID: 8673090

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995 Dec 21-28;378(6559):789-92 https://www.ncbi.nlm.nih.gov/pubmed/8524414?dopt=Abstract PubMed PMID: 8524414