otogenetics® corporation

Patient: ########-COtGx####
CLIA ID#: 11D2066426
Larry Hung, MD, Laboratory Director

Gx™ Hereditary Cancer Risk Assessment Testing Report

Patient Information

Patient Name	xxxx yyyy
Date of Birth	mm dd, yyyy
Age	##
Sex	female
Ethnicity	XXXXXXX

Provider Information

Provider	xxx Women's Center
Provider ID	##########
Physician	xxxx yyyy

Specimen

Accession ID	########
Sample ID	COtGx####
Specimen Type	Saliva
Collection Date	mm dd, 2017
Report Date	mm dd, 2017

Patient Results: Positive - Pathogenic Variant(s) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
PALB2 NM_024675.3: c.758dupT p.S254fs*3	Insertion	Heterozygous	rs515726126; rs756660214	breast cancer	Pathogenic

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
PALB2	4	c.758dupT	p.S254fs*3	rs515726126; rs756660214	Het	Pathogenic

PALB2 is a binding partner of BRCA2 involved in genome maintenance by regulating the DNA damage control pathway. Somatic alterations have been reported in 4.8% of colon cancers, 3.3% of melanomas, and 2.3% of NSCLCs.

Additional Comments

A variant known to be associated with increased risk in hereditary Breast Cancer is detected. The variant in PALB2 gene, c.758dupT (p.S254fs*3), is a frame shift, nonsense mutation that has been observed in familial breast cancer patients (relevant references are included at the end of the report). Clinic correlation is recommended.

Followup Recommendations

Follow up with your healthcare providers for updated genetic risk information. Future findings may provide new clinical interpretation of genetic variants.

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.

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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 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 170721.001), 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 170721.001), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency

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(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. CLIA number – 11D2066426.

Selected Citations

Churpek JE, Walsh T, Zheng Y, Moton Z, Thornton AM, Lee MK, Casadei S, Watts A, Neistadt B, Churpek MM, Huo D, Zvosec C, Liu F, Niu Q, Marquez R, Zhang J, Fackenthal J, King MC, Olopade OI (2014) Inherited predisposition to breast cancer among African American women. Breast Cancer Res Treat. 2015 Jan;149(1):31-9. Epub 2014 Nov 27 https://www.ncbi.nlm.nih.gov/pubmed/25428789?dopt=Abstract PubMed PMID: 25428789

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Wong-Brown MW, Avery-Kiejda KA, Bowden NA, Scott RJ (2013) Low prevalence of germline PALB2 mutations in Australian triple-negative breast cancer. Int J Cancer. 2014 Jan 15;134(2):301-5. Epub 2013 Sep 23 https://www.ncbi.nlm.nih.gov/pubmed/23824750?dopt=Abstract PubMed PMID: 23824750