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CLIA # 11D2065318 | LABSOLUTIONS.COM

Predict™ Hereditary Cancer Risk Assessment Report:

Client: LabSolutions
Client #: 1
Physician Name: Dr. B
Sample ID: 1234567890

Patient: Jane Doe
DOB: 1/1/1955
Gender: Female
Specimen Type: Saliva

Accession #: 1601010001
Collection Date: 1/1/2016
Received Date: 1/2/2016
Report Generated: 1/3/2016

TEST ADMINISTERED:

Predict ™ Hereditary Cancer Risk Assessment. Purification of genomic DNA, sample preparation, enrichment for 39 Inherited Cancer Genes, Illumina HiSeq next generation sequencing, variant filtration as described in Appendix A, clinical interpretation.

CLINICAL INDICATION AND NOTES

Information provided indicates that this individual has a personal and/or family history of cancer.

TEST RESULTS SUMMARY

Primary Findings of genetic testing

Primary Findings:

A rare variation predicted to be pathogenic was identified in the BARD1 gene, namely p.Y678C:c.2033A>G. Clinical significance of this variation is uncertain at this time. BARD1 encodes a protein which interacts with the N-terminal region of BRCA1 (Ref: https://ghr.nlm.nih.gov/gene/BARD1). Mutations in BARD1 were associated with neuroblastoma, ovarian cancer and breast cancer. The inheritance mode of BARD1 is likely dominant.

Variant Name	Associated Phenotype	Variant Function	Exonic Function	Pop Freq	Zygosity	Effect	Chr	Severity
BARD1 c.2033A>G (p.Y678C)	[Gene]:[OMIM]:{Breast cancer, susceptibility to};[ORPHANET]:Hereditary breast and ovarian cancer syndrome;[ClinVar]:Familial cancer of breast;Hereditary cancer-predisposing syndrome;[HGMD]:Colorectal cancer;Neuroblastoma;Ovarian cancer;Breast and/or ovarian	Exonic	Nonsynonymous SNV	0	Hetero-zygous	Unknown	chr2	0.62

Follow up Recommendations

Recommendation for follow up actions based on this screening process include:

- Careful correlation of these variants with the clinical indications and family history for the proband should be conducted by an experienced medical geneticist to verify that reported findings are consistent with reported clinical phenotypes.
- Genetic counseling is recommended for this individual and his/her family to correlate test results with medical and family history, and to fully understand all implications of the test results.
- According to standard ACMG guidelines, sanger validation of any variants of interest is recommended.

Test Statistics

Sample Identifier (Barcode)	Index30_COt0086_062716B
Patient ID	COt0086
Number of DNA reads [number of bases]	4,290,592 [454,802,752]
Targeted capture region	123,000
Average coverage	339
Locations <10X coverage, bases	7

Appendix A

Test Details - Methods and Limitations

Targeted Gene List

Developed by Otogenetics (2015), this gene panel focuses on the 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.

Targeted regions for "Inherited Cancer Gene Panel" includes 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.

Sequencing and Variant Detection

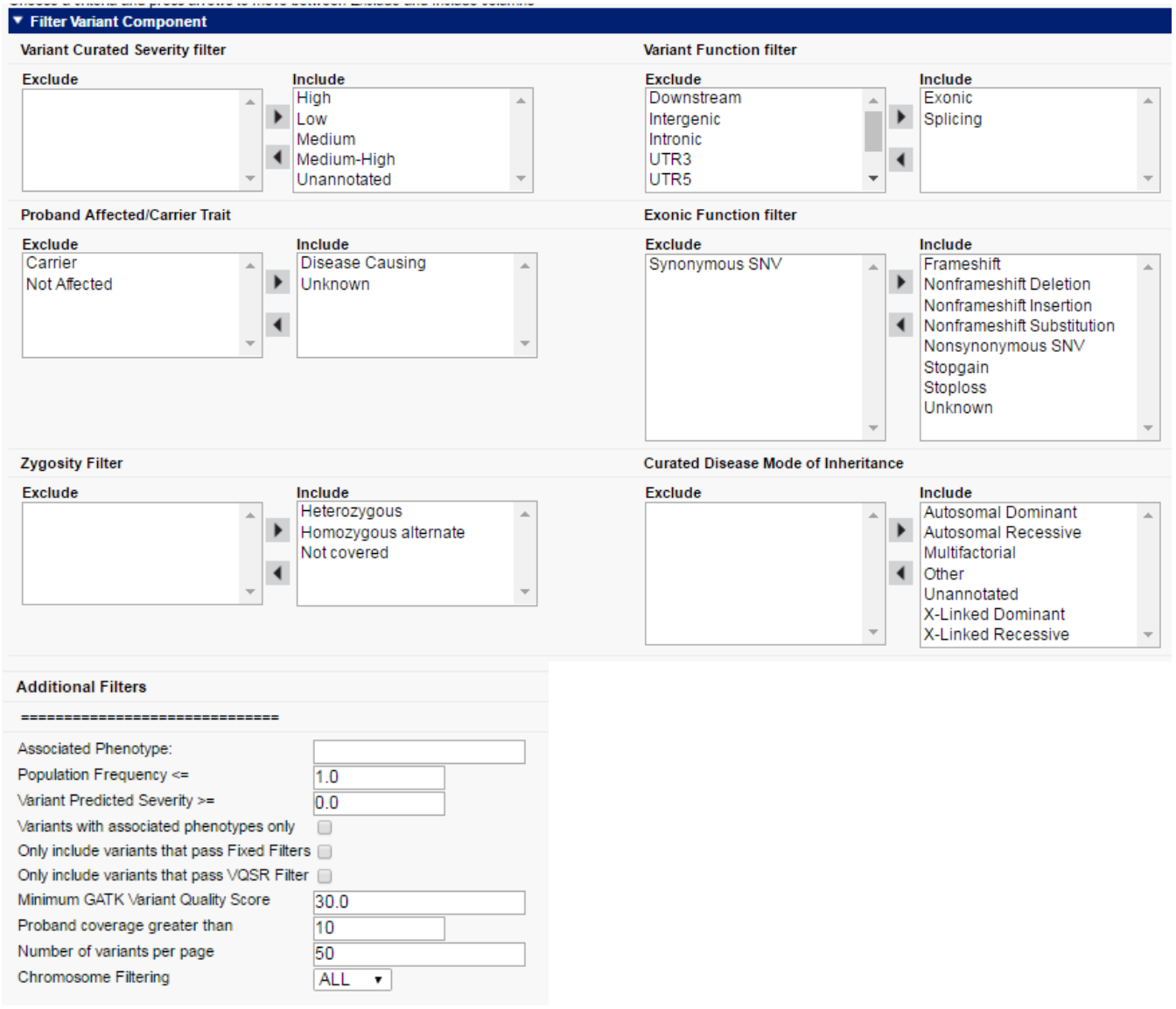
Genomic DNA was extracted from clinical sample (saliva or blood), library preparation via Illumina protocols, capture based enrichment of a targeted region was performed by solution-based hybridization which enriches for coding regions of targeted genes with specific probes. Multiple quality control steps were performed for sample and derivative quality evaluation. Sequencing was performed using the Illumina HiSeq 2500, with 100-125 bp reads, sequence QC metrics were required, and a minimum average coverage depth of 100X was required. Sequencing reads were aligned to the reference genome (UCSC hg19) by BWA-MEM and variants were called using GATK Lite 2.3. The minimum sequence depth for all targeted exons was evaluated; further validation is recommended for exons with depth of coverage <10x. We recommend that variants of interest which do not meet the coverage minimum be confirmed clinically before treatment is undertaken.

Variant Analysis and Clinical reporting

Reported variants were filtered to include those present in the targeted exonic regions and adjacent splice sites. Resulting variants were reported using the Variantyx Genomic Intelligence platform (version 07/01/2016). Annotations based on the following databases were included: HGMD, ClinVar, OMIM, GeneTests, OrphaNet, dbSNP and ensemble. To maintain most up-to-date annotations, the Variantyx database is updated quarterly. As a result, variant classification and/or interpretation may change over time as more information becomes available.

Secondary/Incidental Sequence Variant(s) based on ACMG guidelines are not included in this report.

Not all mutations compared to the reference sequence have been listed on this report. Mutations were identified using the filters described below. These mutations were further reviewed by a medical geneticist, and only variations of clinical significance (primary findings) are included in this report.



References:

The following databases and tools are included in Variantyx Genomic Intelligence platform :

1. Disease association: HGMD Professional (http://www.hgmd.cf.ac.uk/) , Genome Trax (http://www.biobase-international.com/product/genome-trax), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://www.omim.org/), Orphanet (www.orpha.net/), GeneTests (https://www.genetests.org/).
2. Population frequencies: dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), ensemble (www.ensembl.org/), 1000 Genomes Project (www.1000genomes.org/), ExAC (http://exac.broadinstitute.org/), NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and the Variantyx allele frequency database (http://variantyx.com/).
3. Severity predict ion: SIFT, MutationAssessor, Mutation Taster, GWAVA, PolyPhen2, FATHMM, Silva, LRT.
4. Conservation prediction: SiPhy, GERP++, PhyloP and PhastCons.
5. Gene Essentiality: According to published work 10.1371/journal.pgen.1003484
6. Gene tolerance: RVIS score, according to published work 10.1371/journal.pgen.1003709

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. 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.

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.

CLIA Statement

Otogenetics: 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.

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