HGSC Clinical Laboratory
One Baylor Plaza, Houston TX, 77030
Phone: 713.798.6539 Fax: 713.798.5741
www.hgsc.bcm.edu questions@hgsc.bcm.edu

Patient Name:	Jane Nguyen	Sample Collected Date:	11/20/2015
Patient ID:	475-20	Sample Received Date:	12/02/2015
Age:	42	Report Date:	1/12/2016
DOB:	4/28/1974	Sample Type:	DNA
Sex:	Female	Indication for Testing:	Family history of breast cancer
Patient Sample ID:	A4F0G7H5J4	Physician Info:	Dr. Yun Choi
Accession #:	0987654321A		

eMERGE III CAREseq Version 2 Sequencing Report

This test interrogates the protein-coding and exon-splicing regions of 109 genes as well as 1551 single-nucleotide polymorphisms that may impact human health and disease.

Pathogenic Variants

1. **HETEROZYGOUS PATHOGENIC VARIANT IN THE BRCA2 GENE DETECTED:** A heterozygous c.5946delT (p.S1982fs pathogenic variant in the BRCA2 gene was detected in this individual. This variant has been previously reported as disease-causing (PMID 8673091, 15695382, 19188187, 20736950, 22009639, 22703879, 20887823, 21324516, 22430266, 22006311). Defects in BRCA2 are a cause of breast-ovarian cancer, familial, 2 [MIM:612555], breast cancer, male, susceptibility to [MIM:114480], Fanconi anemia, complementation group D1 [MIM:605724], pancreatic cancer [MIM:613347], prostate cancer [MIM:176807] and Wilms tumor [MIM:194070]. This individual is heterozyous for this pathogenic variant, which was confirmed by Sanger sequencing. Clinical correlation and genetic counseling for the patient and at-risk family members is recommended for the pathogenic variant(s).

Table 1: Pathogenic Variant Details:

Disease	Inheritance Pattern	Gene	Position (NCBI 37)	Variant	Zygosity	Notes	MAF	Pathogenicity Prediction
Breast-ovarian cancer, familial, 2 [MIM:612555];	AD	BRCA2	Chr13: 32914437	NM_000059 c.5946delT p.S1982fs Exon11	Heterozygous	Confirmed by Sanger sequencing. PMID 8673091, 22703879, 20736950, 22009639, 15695382, 23633455, 23658460,19188187, 20887823.	N/R	N/A

Likely Pathogenic Variants

1. None Detected

Table 2: Likely Pathogenic Variants Details:

None Detected

Comments & Recommendations:

It is recommended that correlation of these findings with the clinical phenotype be performed. Clinical correlation and genetic counseling for the patient and at-risk family members is recommended for the medically actionable pathogenic variant.

Gene Coverage:

All genes have 100% of targeted bases sequenced to redundant coverage of 20x or greater with the following exceptions: CACNA1B (95.51%), APOB (99.39%), CHEK2 (98.85%), COL5A1 (98.03%), FLG (99.97%), KCNH2 (99.34%), PKP2 (99.77%), RYR1 (98.58%), PMS2 (98.73%), KCNQ1 (90.52%), TGFBR1 (93.56%).

Further information is available in the ExCID report.

Methodology:

1. CAREseq Version 2 NGS Panel*: for the paired-end pre-capture library procedure, genome DNA is fragmented by sonicating genome DNA and ligating to the Illumina multiplexing PE adapters (reference 1). The adapter-ligated DNA is then PCR amplified using primers with sequencing barcodes (indexes). For target enrichment capture procedure, the pre-capture library is enriched by hybridizing to biotin labeled in-solution probes (reference 2) at 56°C for 16 - 19 hours. For massively parallel sequencing, the post-capture library DNA is subjected to sequence analysis on Illumina HiSeq platform for 100 bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads aligned to target, >99% target base covered at >20X, >98% target base covered at >40X, average coverage of target bases >200X. SNP concordance to SNPTrace genotype array: >99%. This test may not provide detection of certain genes or portions of certain genes due to local sequence characteristics or the presence of closely related pseudogenes. Gross deletions or duplications, changes from repetitive sequences may not be accurately identified by this methodology.

2. As a quality control measure**, the individual's DNA is also analyzed by a SNP-array (Fluidigm SNPTrace panel (reference 3)). The SNP data are compared with the NGS panel data to ensure correct sample identification and to assess sequencing quality.

3. Data analysis and interpretation by Mercury 3.4 (reference 3): The output data from Illumina HiSeq are converted from bcl file to FastQ file by Illumina bcl2fastq 1.8.3 software, and mapped by the BWA program (reference 4). The variant calls are performed using Atlas-SNP and Atlas-indel developed in-house by BCM HGSC. Variant annotations are performed using the Cassandra tool, developed in-house. Synonymous variants, intronic variants not affecting splicing site, and common benign variants are excluded from interpretation unless they were previously reported as pathogenic variants. The variants were interpreted according to ACMG guidelines (reference 5) and patient phenotypes. Variants related to patient phenotypes are usually confirmed by Sanger sequencing for patients and if available, parents. Sanger confirmation is noted

in the “Notes” section of the tables if performed. It should be noted that the interpretation of the data is based on our current understanding of genes and variants at the time of reporting.

References:

1. Illumina, Inc. (2011) Multiplexing Sample Preparation Guide (Part # 1005361 Rev. D). 2011.
2. Roche NimbleGen, Inc. (2010) NimbleGen SeqCap EZ Exome Library SR User’s Guide (Version 2.2).
3. Liang-Chu MM, Yu M, Haverty PM, Koeman J, Ziegle J, Lee M, Bourgon R, Neve RM. Human biosample authentication using the high-throughput, cost-effective SNPtrace(TM) system. PLoS One. 2015 Feb 25;10(2):e0116218.
4. Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60. PMID:19451168.
5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. 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 (2015) 17, 405-423. PMID: 25741868.

Christine M. Eng, M.D.
Medical Director
May 4, 2016

Yaping Yang, Ph.D
Laboratory Co-Director
May 4, 2016

This test was developed and its performance determined by this laboratory. It has not been cleared or approved by U.S. Food and Drug Administration. Since FDA is not required for clinical use of this test, this laboratory has established and validated the test's accuracy and precision, pursuant to the requirement of CLIA '88. This laboratory is licensed and/or accredited under CLIA and CAP (CAP# 8004250 / CLIA# 45D2027450).