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Excellence In Health Care

DEPARTMENT OF MOLECULAR GENETICS AND GENOMICS

Patient Name	Mrs. Santilata Mishra	Ref. Doctor	Self
Age/Gender	62Y / FEMALE	Ref. Hospital	-
Barcode	25092306	Sample Type	Whole Blood EDTA

Clinical History:

Mrs. Santilata Mishra presented with complaints of lower abdominal pain for 12 months. She is diagnosed with high grade serous carcinoma, right ovary, recurrence post TAH BSO-FIGO stage IIB. CECT- thorax and abdomen showed a well-defined round predominantly cystic lesion measuring ~6.8x6.3x6.6vm (AP*TR*CC*) with an enhancing solid component measuring 1.5x2.3cm in posterior wall of the lesion noted in the pelvis, A 1x1cm presacral node noted, likely metastatic / deposit, uterus and bilateral ovaries not visualized. She was referred for *BRCA1* and *2* germline sequencing.

Results: No Pathogenic or Likely Pathogenic variants identified in *BRCA1* and *BRCA2* genes related to the individual's phenotype.

Interpretation:

The sequence analysis revealed no significant pathogenic or likely pathogenic variants associated to the patient's clinical phenotype. The absences of pathogenic mutations do not rule out the patient's disease condition. Hence, additional testing is recommended.

Recommendations:

- Genetic counselling is recommended.

Test Information

The total genomic DNA was extracted from the biological sample using the column-based method and DNA quality and quantity were assessed using electrophoretic and Qubit methods. The QC-qualified genomic DNAwas randomly fragmented and ligating sequencing adapters were added to both ends of DNA fragments. Sequencing libraries were size-selected using beads to optimal template size and amplified by polymerase chain reaction. The regions of interest (exons and flanking intronic targets) are targeted by a hybridization-based target capture method. Sequencing libraries that passed the quality control were sequenced on the MGI platform using paired-end chemistry. Reads were assembled and are aligned to reference sequences based on NCBI Ref Seq transcripts and human genome build GRCh38. Data was filtered and analyzed to identify variants of interest related to patients' clinical phenotype.

Tools and databases used for data analysis:

We followed the Genome Analysis Toolkit (GATK) best practices framework for the identification of variants in the sample. The sequences obtained were subjected to quality assessment and pre-processing. The pre-processed sequences were aligned with human reference genome sequence (assembly GRch38) by Burrows-Wheeler Aligner and post-alignment processing like read duplicate removal and base quality score recalibration (BQSR) was carried out by using GATK (v4.2.5.0). Variant calling was done by using the GATK Haplotype Caller. Each called variant is

annotated using different clinical and population databases. Common variants were filtered out based on minor allele frequency (MAF) in 1000Genome Phase 3[4], gnomAD (v4), ExAC [3], and dbSNP (v155). Non-synonymous variants effect is calculated using multiple in-silico algorithms. Only non-synonymous and splice site variants with clinical relevance were selected using published literature and a set of disease databases -ClinVar, OMIM, GWAS, and SwissVar. The classification of the variant is done based on American College of Medical Genetics guidelines.

QC METRICS

Total data generated	5.03Gb
Total reads aligned reads (%)	90.54%
Data ≥Q30(%)	91.48%
Total data which passed mapping quality cut-off	4.51Gb

Variant or Mutation	The change(s) in a gene. This could be disease-causing (pathogenic) or non-disease-causing (benign).
Pathogenic	A disease-causing mutation in a gene has been identified, which may explain or correlate with the patient's symptoms. This usually denotes the confirmation of a suspected condition for which testing was requested.
Likely Pathogenic	A variant that is very likely to play a role in the development of disease, but currently scientific evidence is inadequate, additional evidence in the future may declare the pathogenicity of this variant.
Variant of Uncertain Significance	A variant has been identified, but existing scientific information makes it impossible to define as pathogenic (disease-causing) or benign (non-disease-causing) and further required functional studies. The clinician may recommend additional tests for the patient or family members. It is likely that their relevance will only be determined over time, depending on the availability of scientific information.

Test Limitations:

A negative or normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate/incomplete information may lead to misinterpretation of the results.

Test Attributes:

- It is presumed that the specimen used to perform the test belongs to the patient specified above, such verification having been carried out at the collection level of the sample.
- The current results are based on analysis of coding regions (exons) as well as certain intron padding regions on the patient's genomic DNA with respect to patient phenotype as defined in the target regions (link available below). However, due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants of insufficient coverage, as well as those variants that currently do not correlate with the provided phenotype may not be analysed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- The reported variants have not been Sanger confirmed. Sanger confirmation is recommended for the same.

- The test methodology currently does not detect large deletions/duplications, triplet repeat expansions, and epigenetic changes. The test also does not include an analysis of predictors for multifactorial, polygenic, and/or complex diseases. Novel synonymous changes as well as intronic mutations (excludingthoseaffecting invariant splice nucleotides) are not routinely reported.
- CNV analysis is not included.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity & specificity for variant detection, analysis, and interpretation due to the inability of the data tools to unambiguously determine the origin of the sequence data. The mutations have not been validated by Sanger sequencing unless specified.
- Regions other than the targeted are not covered and hence cannot be reported.
- Phenotype variability may be due to modifying genetic/non-genetic factors and is not a part of the current analysis.
- This test has not been validated by the FDA, NABL, or CAP, and it has been determined by the accrediting bodies that such validation is not required atthis time.
- In some instances, the classification and interpretation of variants (VUS) may change as new scientific information comes to light. We recommend a re-analysis of this report yearly. Please contact the laboratory in case a re-analysis of the report is desired. It is the lab's policy to perform re-analysis once on a complimentary basis. However, this re-analysis is performed only when requested.

Gene coverage:

Gene	% Covered (30X)
BRCA1	75.20
BRCA2	88.97

References

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