Hereditary Cancer Test

MC-2434

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Patient Name	:	**Pradeepa Dissanayake**	Test	:	**Hereditary BRCA1/BRCA2 Test**
Gender	:	**Female**	Referring Center	:	**Aegle Omics Private Limited**
Age	:	**53 Years**	Referred by	:	**Dr. Mahendra Perera**
MRN #	:	**NA**	Sample Collected	:	**09-Sep-2024**
Sample ID	:	**STRAN-2024-58021**	Sample Received	:	**12-Sep-2024**
Specimen	:	**Blood**	Report Generated	:	**10-Oct-2024**

Indications for Test

Diagnosed with ovarian clear cell adenocarcinoma.

Results

NEGATIVE* — No disease-causing or likely disease-causing variant was detected in the genes (as mentioned below) tested in this sample.

Test Details

This test analyzes 2 genes (*BRCA1* and *BRCA2*) associated with hereditary cancer predisposition.

Please refer to the 'limitations of gene coverage' section for the list of genes showing low coverage (<20 reads) in this sample.

Note

- *This is an amended (revised) version-2 report.*
- *In the version-1 report, generated on 09-Oct-2024, the referring physician's name was incorrectly mentioned in the page 1 under the demographics section of the report.*
- *The physician's name has been corrected and a version-2 report has been issued.*

Interpretation Summary

- No germline pathogenic (disease-causing) or likely pathogenic (likely disease-causing) variant in the 2 genes (*BRCA1* and *BRCA2*) associated with hereditary cancer predisposition has been identified in this individual.
- A negative test result does not exclude the possibility that this individual's personal history of cancer has a genetic cause, as it may be due to variation in a genomic region not covered by the test or it can also be due to the inherent technical limitations of the test.

Recommendations

- Genetic counseling is recommended to discuss the implications of this test result for this individual.
- The physician can request reanalysis of the data and this is recommended on an annual basis. Data from this test is based on currently available scientific information. This data can be re-assessed for the presence of

**When an affected individual tests negative, then the result can be classified as an 'uninformative negative' (that is, does not provide useful information). It is possible that an individual truly does not harbor a 'disease-causing' variant in the tested genes or it could be due to inherent technical limitations of the test. Refer to low coverage table and the 'Limitations and Disclaimer' sections for more information.*

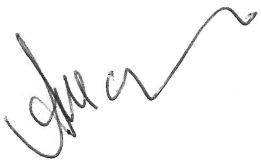
any variants that may be newly linked to established genes associated with the hereditary cancer or to newly identified disorders since the date of this report. A charge may apply for reanalysis.

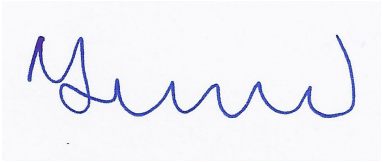
- For further details, kindly contact: report.strandx@strandls.com

Limitations of Gene Coverage

For each test gene, all gene target regions were adequately covered by greater than 20 reads.

Signatures





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Supplementary Information

Test Description

The Hereditary Cancer Test includes genes associated with commonly inherited cancers (breast, colorectal, endocrine, gynaecological, melanoma, nervous system, pancreatic and prostate) as well as several rare cancers. The test involves preparation of a target sequence enrichment based library from the patient's genomic deoxyribonucleic acid (gDNA) using Roche's KAPA HyperPlus technology. The test covers unique genes associated with hereditary cancers and includes variation coverage for all coding exons (>97%) and essential splice sites. The generated library is subjected to next generation sequencing (NGS) on the Illumina NGS platform (NovaSeq/NextSeq). Genetic variations are identified by using the STRAND NGS software and interpreted using the StrandOmics platform.

The Hereditary Cancer Test is a Laboratory Developed Test (LDT) that was developed and its performance validated by Strand Life Sciences Pvt. Ltd.

Genes Evaluated: 2 genes

BRCA1, BRCA2

Methodology

Library preparation: Genomic DNA isolated from saliva, blood or any other standard tissue source is used for preparation of the 'DNA sequencing ready' library. The DNA is quantified using Qubit Fluorometer and 50 ng is taken for library preparation. The "KAPA HyperPlus kit" is used to prepare the DNA sequencing libraries. The KAPA HyperPlus protocol allows the DNA to be "fragmented" to produce dsDNA fragments. An end repair and Atailing step produce end-repaired dsDNA fragments. An adapter ligation step produces an adapter-ligated 3'-dA-tailed dsDNA molecule. In addition, a high-fidelity, low-bias library amplification step allows the incorporation of platform-specific tags and barcodes to prepare the DNA sequencing libraries.

Target Enrichment: The tagged and amplified sample libraries are checked for quality and quantified. Individual libraries are combined into a single tube and set up for enrichment. Two simultaneous enrichment steps are performed to optimize the pull-down of the regions of interest using biotinylated target-specific probes using a commercial kit (IDT, USA). Target libraries are amplified using limited PCR steps and loaded for sequencing on the NovaSeq/NextSeq instrument.

Sequencing Details: Sequencing is performed on Illumina NovaSeq/NextSeq instruments. For this test, the expected output is ≥ 0.3 GB per sample.

Analysis

The reads from the FASTQ files were aligned against the whole genome build hg19 using STRAND NGS v3.3.5 (http://www.strand-ngs.com) via the analysis pipeline 'GlcT1_Trusight_GermlineCancer_Production_v9'. The reads were aligned against the whole genome build hg19 using STRAND NGS (http://www.strand-ngs.com). Five base pairs from the 3' end of the reads were trimmed, as were 3' end bases with quality below 20. Reads which had length less than 25 bp after trimming were not considered for alignment. A maximum of 5 matches of alignment score at least 90% with a gap percentage of 45 were computed. Reads that failed QC (quality control), reads with average quality less than 20, reads with ambiguous characters and reads which are duplicates were all filtered out. The reads were realigned using the local realignment tool in STRAND NGS. The reads with alignment score less than 95% and partially aligned reads were all filtered out. The STRAND NGS variant caller was used to detect variants at locations in the target regions covered by a minimum of 10 reads with at least 2 variant reads. Mate missing reads were ignored while calling the variants. Variants with a decibel score of at least 50 were reported. Variants with a Strand Bias ≥100% and with a Total Reads ≥50, and InDel variants in homopolymer stretches ≥12 bp long with supporting reads ≤90% were filtered out. Variants were then imported into StrandOmics. Annotation and prioritization of variants was done by automated pipelines in StrandOmics. The StrandOmics user interface was then used for identifying variants of interest and for reporting these variants. All variants reported were verified to have good raw read quality using the STRAND NGS genome browser.

CNV (Copy Number Variation) Analysis: In addition to SNVs and small Indels, copy number analysis was performed by comparing the normalized coverage of the panel regions against a profile created from multiple samples sequenced in the run. This analysis was also performed in STRAND NGS.

SV (Structural Variation) Analysis: SV analysis is also performed on the read data. The output of local realignment is fed to this step as input. Also hard clipped reads are filtered out before performing the SV detection. Additional filters are applied on output of SV detection to eliminate potential false positives.

Analytical performance: Analytical validation (Document No.: VR074) of this test done in our laboratory has shown sensitivity of 100%, specificity of 99.9% and reproducibility of 100%.

STRAND NGS v3.3.5: STRAND NGS (http://www.strand-ngs.com) is an NGS analysis platform from Strand Life Sciences. It comprises algorithms for alignment, variant calling, exon deletion/duplication analysis, and structural variant calling. A built in genome browser enables inspection of read level data. Several QC steps enable inspection of read quality. STRAND NGS has been cited in at least 200 publications.

StrandOmics v6.25.0: StrandOmics is a clinical genomics interpretation and reporting platform from Strand Life Sciences. The StrandOmics Variant Annotation engine includes algorithms to identify variant impact on gene using both public content (ClinVar, HPO, links to dbSNP, 1000

Genomes, Exome Variant Server, and five in-silico predictors - FATHMM, LRT, Mutation Assessor, Mutation Taster, and SIFT) and proprietary content (curated variant records). The interpretation interface in StrandOmics allows quick filtering and evaluation of variants along with capture of justification for inclusion/exclusion. The reporting interface in StrandOmics enables included variants to be carried into template-driven reports efficiently. StrandOmics has already been used for interpretation of thousands of clinical cases.

Data Versions: Data Annotations are updated periodically. Data version or date of download used for annotations are as mentioned. Human Genome (hg19), NCBI RefSeq (Annotation Release 105), NCBI RefSeq genes-curated subset (Apr 2018), NCBI Gene (June 2018), ClinVar (June 2023), UniProt (June 2023), dbSNP (v156), Exome Variant Server (Jan 2017), 1000 Genomes (Jan 2017), dbNSFP (v2.9.3), HPO (Aug 2023).

Limitations and Disclaimer

As with any laboratory test, there is a small chance that this result may be inaccurate for a procedural reason, such as an error during specimen collection and labeling (incorrect patient identification), an error in processing, data collection, or interpretation. Currently available data indicates that technical error rate for analysis involving DNA tests is anywhere between 1-3%. Variants that have not been confirmed by an independent analysis could represent technical artifacts. However, our validation study (Document No. VR-017v1) showed 100% concordance between the results obtained by NGS data and Sanger sequencing (confirmation of the variant), when the supporting read fraction of the variant with atleast 20 reads was >30%. Large insertions, deletions, duplications, inversions, repeat expansions and complex rearrangements cannot be characterized accurately by NGS as it uses short-read sequencing data. Such structural variants have a much higher false-positive and false-negative rate than seen for SNVs (single nucleotide variant). It is possible that the genomic region where a disease causing variation exists in the proband (which may impact the phenotype) was not captured using the current technologies and therefore was not detected. For an autosomal dominant condition, if the variant does not seem to be inherited from parents, it could be due to a *de novo* (new) event or due to a germline mosaicism in an unaffected parent. In case of germline mosaicisim, there is a risk of the disease recurrence in the family. However, due to technical limitation of this test, germline mosaicism cannot be determined by this test. Additionally, 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 on those genes. Not all variations detected may be listed in the report. Inclusion of variations is dependent upon our assessment of their significance. The quality of sequencing and coverage varies between regions. Specific genomic regions, such as homopolymers, tandem repeat sequence, GCrich regions, high sequence homology, etc. influence the quality of sequencing and coverage. This may result in an occasional error in sequence reads or lack of detection of a particular genetic lesion. Accurate interpretation of this report is dependent on detailed clinical history of the patient. In the event of unavailability of detailed clinical history, the lab cannot guarantee the accuracy of the interpretation. This report is strictly not a medical diagnostic report and shall not be construed as the medical certificate or medical laboratory report or diagnostic report.

Compliance Statement

This assay was developed and its performance validated by Strand Life Sciences Pvt. Ltd. This laboratory is following the regulatory requirements and guidelines of College of American Pathologists (CAP). Genetic counselling is recommended for all patients undergoing genetic testing. We follow the American College of Medical Genetics and Genomics (ACMG) guidelines regarding guidelines for test validation, variant classification and reporting [1-8].

Supplementary Information - References

1. Rehm HL *et al.* 2013. ACMG clinical laboratory standards for next-generation sequencing. *Genet. Med.* **15** (9):733-47 [PMID: 23887774].
2. Richards S *et al.* 2015. 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. *Genet Med* **17** (5):405-424 [PMID: 25741868].
3. American College of Medical Genetics and Genomics 2013. Incidental findings in clinical genomics: a clarification. *Genet. Med.* **15** (8):664-6 [PMID: 23828017].
4. Hirschhorn K *et al.* 1999. Duty to re-contact. *Genet. Med.* **1** (4):171-2 [PMID: 11258354].
5. Lubin IM *et al.* 2008. Ordering molecular genetic tests and reporting results: practices in laboratory and clinical settings. *J Mol Diagn* **10** (5):459-68 [PMID: 18669879].
6. Lubin IM *et al.* 2009. Clinician perspectives about molecular genetic testing for heritable conditions and development of a clinician-friendly laboratory report. *J Mol Diagn* **11** (2):162-71 [PMID: 19197001].
7. Plon SE *et al.* 2008. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum. Mutat.* **29** (11):1282-91 [PMID: 18951446].
8. Richards CS *et al.* 2008. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet. Med.* **10** (4):294-300 [PMID: 18414213].

End of report