MOLECULAR BIOLOGY

BRCA 1 & 2 Full Gene Sequencing (with Sanger validation) *(Whole Blood)*

Result .

Test Name: BRCA Next

Test Information:

This test uses Next Generation Sequencing (NovaSeq 6000), targeted sequencing approach that is restricted to the protein coding regions of selected genes under investigation. BRCA1 and BRCA2 Panel contains primer pairs that target the coding regions of the tumor suppressor genes BRCA1 and BRCA2, which have been implicated in hereditary breast and ovarian cancers. This panel utilizes 167 amplicons to analyse the coding region of both BRCA1 and BRCA2 genes.

Sample Type:

EDTA Whole Blood

Clinical Information & Family History:

Adenocarcinoma of ovary

Results:

NEGATIVE

(No pathogenic variant detected related to the clinical phenotype)

Name of Gene	Variant Details	Clinical Significance
BRCA1	None	-
BRCA2	None	-

Recommendation:

- We recommend confirming the presence of variants by Sanger Sequencing.
- BRCA1 & BRCA2 MLPA study is recommended to analyse deletion/duplications in the sample.
- The results should be interpreted in the context of the patient's medical evaluation. Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis, prognosis or for therapeutic decisions.
- The classification of variants of unknown significance (VUS) can change over time.

*Genetic test results are reported based on the recommendations of American College of Medical Genetics (ACMG).

Variant	A change in a gene. This could be disease-causing (pathogenic) or not disease-causing (benign).
Pathogenic	A disease-causing variation in a gene that can explain the patient's symptoms has been detected.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease, however, the scientific evidence is currently insufficient to prove this conclusively.
Uncertain clinical significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease-causing) or benign (non-disease causing) based on currently available scientific evidence.
Likely Benign	A variant is not expected to have a major effect on disease.
Benign	A variant which is known not to be responsible for the disease has been detected.

Test Summary:

BRCA1: This gene encodes a 190 kD nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor. The BRCA1 gene contains 22 exons spanning about 110 kb of DNA. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the subcellular localization and physiological function gene. Many alternatively spliced transcript variants, some of which are disease-associated mutations, have been described for this gene, but the full-length natures of only some of these variants has been described. A related pseudogene, which is also located on chromosome 17, has been identified (Gene:672).

BRCA2: Inherited mutations in BRCA1 and this gene, BRCA2, confer an increased lifetime risk of developing breast or ovarian cancer. Both BRCA1 and BRCA2 are involved in the maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. The largest exon in both genes is exon 11, which harbours the most important and frequent mutations in breast cancer patients. The BRCA2 gene was found on chromosome 13q12.3 in humans. The BRCA2 protein contains several copies of a 70 aa motif called the BRC motif, and these motifs mediate binding to the RAD51 recombinase which functions in DNA repair. BRCA2 is considered a tumor suppressor gene, as tumors with BRCA2 mutations generally exhibit loss of heterozygosity (LOH) of the wild-type allele (Gene: 675).

Test Methodology:

- BRCA1 & BRCA2 Panel on Next Generation Sequencing (NovaSeq 6000) is a targeted sequencing approach that is restricted to the protein-coding regions of selected genes under investigation.
- BRCA1 and BRCA2 Panel contains primer pairs that target the coding regions of the tumor suppressor genes BRCA1 and BRCA2, which have been implicated in breast and ovarian cancers. This panel utilizes 167 amplicons to analyse the coding region of both BRCA1 and BRCA2 genes.
- DNA isolated from Peripheral Blood or Buccal Swab is used for NGS Library preparation.
- The libraries were sequenced to mean depth: >150x on next generation sequencing platform.
- The raw read sequences obtained from NGS are processed to remove adapters and filter poor quality reads.
- Clinically relevant germline mutations were identified and annotated using published variants in literature and a set of diseases databases.
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- The effect of non-synonymous variant is calculated using multiple prediction algorithms such as PolyPhen, SIFT, Mutation Taster2.

Test Limitations & Disclaimer:

- This test is limited to BRCA1 & BRCA2 genes analysis only, It should be noted that this test does not sequence all bases in a human genome, not all variants have been identified or interpreted, and this report is limited only to variants with evidence for causing or contributing to disease/clinical details provided.
- This assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications.

References:

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3. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. Journal of Clinical Oncology 2007; 25(11):1329–1333.

4. Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. Journal of the National Cancer Institute 2002; 94(18):1365–1372.

5. Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 mutation. JAMA 2006; 296(2):185–192.

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7. Tai YC, Domchek S, Parmigiani G, Chen S. Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. Journal of the National Cancer Institute 2007; 99(23):1811–1814.

#Test performed at refer

Disclaimer:
1. All results released pertain to the specimen as received by the lab for testing and under the assumption that the patient indicated or identified on the bill/test requisition form is the owner of the specimen.
2. Clinical details and consent forms, especially in Genetic testing, histopathology, as well as wherever applicable, are mandatory to be accompanied with the test requisition form. The non-availability of such information may lead to delay in reporting as well as misinterpretation of test results. The lab will not be responsible for any such delays or misinterpretations thereof.
3. Test results are dependent on the quality of the sample received by the lab. In case the samples are preprocessed elsewhere (e.g., paraffin blocks), results may be compromised.
4. Tests are performed as per the schedule given in the test listing and in any unforeseen circumstances, report delivery may be affected.
5. Test results may show inter-laboratory as well as intra-laboratory variations as per the acceptable norms.
6. Genetic reports as well as reports of other tests should be correlated with clinical details and other available test reports by a qualified medical practitioner. Genetic counselling is advised in genetic test reports by a qualified genetic counsellor, medical practitioner or both.
7. Samples will be discarded post processing after a specified period as per the laboratory's retention policy. Kindly get in touch with the lab for more information.
8. If accidental damage, loss, or destruction of the specimen is not attributable to any direct or negligent act or omission on the part of Ampath Labs or its employees, Ampath shall in no event be liable. Ampath lab's liability for a lack of services, or other mistakes and omissions, shall be restricted to the amount of the patient's payment for the pertinent laboratory services.