| Patient Name | DOB | Sex | MRN | Invitae # |
|---|---|---|---|---|
| Jane Doe | | Female | | |

| Clinical Team | Report Date | Sample Type | Sample Collection Date | Sample Accession Date |
|---|---|---|---|---|
| | | Blood | | |

**Test Performed**
Sequence analysis and deletion/duplication testing of the 43 genes listed in the results section below.
- Invitae Breast and Gyn Cancers Panel
- Invitae Common Hereditary Cancers Panel

**Reason for Testing**
Diagnostic test for a personal and family history of disease

## Summary

# Positive result. Pathogenic variant identified in BRCA1.

## Clinical Summary

- A Pathogenic variant, Deletion (Exon 20), was identified in BRCA1.
  - The BRCA1 gene is associated with autosomal dominant hereditary breast and ovarian cancer (HBOC) syndrome (MedGen UID: 151793).
  - This result is consistent with a predisposition to, or diagnosis of, BRCA1-related conditions.
  - HBOC syndrome is characterized by an increased lifetime risk for breast cancer, contralateral breast cancer, male breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, and other cancers (PMID: 12237281). Lifetime risk estimates in females with a pathogenic variant in BRCA1 include 40-87% risk of breast cancer, up to 43% risk of contralateral breast cancer within 10 years of the initial primary, and 16-54% risk of ovarian, fallopian tube, or peritoneal cancer (PMID: 9497246, 12677558, 10498392, 14576434, 9145676, 15197194). The risk for breast cancer in males with a pathogenic variant in BRCA1 is 1-2% (PMID: 20587410, 18042939). There are also increased risks for prostate cancer (20%), and pancreatic cancer (1-3%) (PMID: 10433620, 22187320, 7907678, 23099806). Clinical management guidelines for HBOC syndrome can be found at www.nccn.org.
  - Close relatives (children, siblings, and parents) have up to a 50% chance of being a carrier of this variant. More distant relatives may also be carriers. Parental testing may clarify the inheritance of this variant and may inform recurrence risk and risk for other close relatives. Testing for this variant is available.
- These results should be interpreted within the context of additional laboratory results, family history, and clinical findings. Genetic counseling is recommended to discuss the implications of this result. For access to a network of genetic providers, please contact Invitae at clientservices@invitae.com, or visit www.nsgc.org or tagc.med.sc.edu/professional_organizations.asp.

## Complete Results

| Gene | Variant | Zygosity | Variant Classification |
|---|---|---|---|
| BRCA1 | Deletion (Exon 20) | heterozygous | PATHOGENIC |

The following genes were evaluated for sequence changes and exonic deletions/duplications:
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A (p14ARF), CDKN2A (p16INK4a), CHEK2, DICER1, EPCAM (Deletion/duplication testing only), GREM1 (Promoter region deletion/duplication testing only), KIT, MEN1, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL

The following genes were evaluated for sequence changes only:
HOXB13 (c.251G>A, p.Gly84Glu variant only), SDHA

Results are negative unless otherwise indicated

Benign, Likely Benign, and silent and intronic variants with no evidence towards pathogenicity are not included in this report but are available upon request.

## Variant Details

BRCA1, Deletion (Exon 20), heterozygous, PATHOGENIC

- This variant is an out-of-frame deletion of the genomic region encompassing exon 20 of the BRCA1 gene. This is expected to create a premature translational stop signal and result in an absent or disrupted protein product.
- This variant has not been reported in the literature in individuals with BRCA1-related disease.
- Loss-of-function variants in BRCA1 are known to be pathogenic (PMID: 20104584).
- For these reasons, this variant has been classified as Pathogenic.

## Methods

- Genomic DNA obtained from the submitted sample is enriched for targeted regions using a hybridization-based protocol, and sequenced using Illumina technology. Unless otherwise indicated, all targeted regions are sequenced with ≥50x depth or are supplemented with additional analysis. Reads are aligned to a reference sequence (GRCh37), and sequence changes are identified and interpreted in the context of a single clinically relevant transcript, indicated below. Enrichment and analysis focus on the coding sequence of the indicated transcripts, 10bp of flanking intronic sequence (20bp for BRCA1/2), and other specific genomic regions demonstrated to be causative of disease at the time of assay design. Promoters, untranslated regions, and other non-coding regions are not otherwise interrogated. For some genes only targeted loci are analyzed (indicated in the table above). Exonic deletions and duplications are called using an in-house algorithm that determines copy number at each target by comparing the read depth for each target in the proband sequence with both mean read-depth and read-depth distribution, obtained from a set of clinical samples. All clinically significant observations are confirmed by orthogonal technologies, except individually validated variants and variants previously confirmed in a first-degree relative. Confirmation technologies include any of the following: Sanger sequencing, Pacific Biosciences SMRT sequencing, MLPA, MLPA-seq, Array CGH. Array CGH confirmation of NGS CNV calling performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778). The following analyses are performed if relevant to the requisition. For PMS2 exons 12-15, the reference genome has been modified to force all sequence reads derived from PMS2 and the PMS2CL pseudogene to align to PMS2, and variant calling algorithms are modified to support an expectation of 4 alleles. If a rare SNP or indel variant is identified by this method, both PMS2 and the PMS2CL pseudogene are amplified by long-range PCR and the location of the variant is determined by Sanger sequencing of the relevant exon in both long-range amplicons. If a CNV is identified, MLPA or MLPA-seq is run to confirm the variant. If confirmed, both PMS2 and PMS2CL are amplified by long-range PCR, and the identity of the fixed differences between PMS2 and PMS2CL are Sanger sequenced from the long-range amplicon to disambiguate the location of the CNV. Technical component of confirmatory sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778).
- The following transcripts were used in this analysis: APC (NM_000038.5), ATM (NM_000051.3), AXIN2 (NM_004655.3), BARD1 (NM_000465.3), BMPR1A (NM_004329.2), BRCA1 (NM_007294.3), BRCA2 (NM_000059.3), BRIP1 (NM_032043.2), CDH1 (NM_004360.3), CDKN2A (p14ARF) (NM_058195.3), CDKN2A (p16INK4a) (NM_000077.4), CHEK2 (NM_007194.3), DICER1 (NM_177438.2), EPCAM (NM_002354.2: Deletion/duplication testing only), GREM1 (NM_013372.6: Promoter region deletion/duplication testing only), HOXB13 (NM_006361.5: c.251G>A, p.Gly84Glu variant only), KIT (NM_000222.2), MEN1 (NM_130799.2), MLH1 (NM_000249.3), MSH2 (NM_000251.2), MSH6 (NM_000179.2), MUTYH (NM_001128425.1), NBN (NM_002485.4), NF1 (NM_000267.3), PALB2 (NM_024675.3), PDGFRA (NM_006206.4), PMS2 (NM_000535.5), POLD1 (NM_002691.3), POLE (NM_006231.3), PTEN (NM_000314.4), RAD50 (NM_005732.3), RAD51C (NM_058216.2), RAD51D (NM_002878.3), SDHA

(NM_004168.3), SDHB (NM_003000.2), SDHC (NM_003001.3), SDHD (NM_003002.3), SMAD4 (NM_005359.5), SMARCA4 (NM_001128849.1), STK11 (NM_000455.4), TP53 (NM_000546.5), TSC1 (NM_000368.4), TSC2 (NM_000548.3), VHL (NM_000551.3).

- A PMID is a unique identifier referring to a published, scientific paper. Search by PMID at http://www.ncbi.nlm.nih.gov/pubmed.
- An rsID is a unique identifier referring to a single genomic position, and is used to associate population frequency information with sequence changes at that position. Reported population frequencies are derived from a number of public sites that aggregate data from large-scale population sequencing projects, including ExAC (http://exac.broadinstitute.org) and dbSNP (http://ncbi.nlm.nih.gov/SNP).
- A MedGen ID is a unique identifier referring to an article in MedGen, NCBI's centralized database of information about genetic disorders and phenotypes. Search by MedGen ID at http://www.ncbi.nlm.nih.gov/medgen. An OMIM number is a unique identifier referring to a comprehensive entry in Online Mendelian Inheritance of Man (OMIM). Search by OMIM number at http://omim.org/.

## Limitations

- This assay achieves >99% sensitivity and specificity for single nucleotide variants and insertions and deletions <15bp indels, based on validation study results. Sensitivity to detect insertions and deletions larger than 15bp but smaller than a full exon may be marginally reduced. Expansions and contractions within trinucleotide repeat regions may not be detected unless specified. Invitae's deletion/duplication analysis determines copy number with high confidence at >95% of targeted exons. This methodology may not detect low-level mosaicism. This report reflects the analysis of an extracted DNA sample. In very rare cases, (circulating hematolymphoid neoplasm, bone marrow transplant, recent blood transfusion) the analyzed DNA may not represent the patient's constitutional genome. IDS: Detection of complex rearrangements not offered (PMID: 7633410, 20301451).

**This report has been reviewed and approved by:**

**Placeholder for signature**

## Disclaimer

DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it is not a diagnosis itself. This test was developed and its performance characteristics determined by Invitae. It has not been cleared or approved by the FDA. The laboratory is regulated under the Clinical Laboratory Improvement Act (CLIA) as qualified to perform high-complexity clinical tests (CLIA ID: 05D2040778). This test is used for clinical purposes. It should not be regarded as investigational or for research.

**Laboratory Director** Swaroop Aradhya, Ph.D., FACMG
**Invitae** 1400 16th Street, San Francisco, CA 94103
**E:** clientservices@invitae.com **P:** 415.374.7782 or 800.436.3037