INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

Patient name:	John Doe	**Sample type:**	Blood	**Report date:**	
DOB:		**Sample collection date:**		**Invitae #:**	
Sex:	Male	**Sample accession date:**		**Clinical team:**	
MRN:					

Reason for testing

Diagnostic test for a personal history of disease

Test performed

Sequence analysis and deletion/duplication testing of the 19 genes listed in the Genes Analyzed section.

- Invitae Prostate Cancer Panel
- Add-on Preliminary-evidence Genes for Prostate Cancer

RESULT: POSITIVE

One Pathogenic variant identified in BRCA2. BRCA2 is associated with autosomal dominant hereditary breast and ovarian cancer syndrome and autosomal recessive Fanconi anemia.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
BRCA2	c.8633-1G>A (Splice acceptor)	heterozygous	PATHOGENIC

About this test

This diagnostic test evaluates 19 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Next steps

- This is a medically important result that should be discussed with a healthcare provider, such as a genetic counselor, to learn more about this result and the appropriate next steps for further evaluation, treatment and/or management. This result should be interpreted within the context of additional laboratory results, family history and clinical findings.
- Please see NCCN (www.nccn.org) for management guidelines regarding BRCA2-related condition(s).
- Consider sharing this result with relatives as they may also be at risk. Details on our Family Variant Testing program can be found at www.invitae.com/family.
- Register your test at www.invitae.com/patients to download a digital copy of your results. You can also access educational resources about how your results can help inform your health.

Laboratory Director: Kristin Gibson, PhD, FACMG, NYCQ, CGMB, ErCLG
Invitae 1400 16th Street, San Francisco, CA 94103 · clientservices@invitae.com · 800.436.3037

Clinical summary

A Pathogenic variant, c.8633-1G>A (Splice acceptor), was identified in BRCA2.

- The BRCA2 gene is associated with autosomal dominant hereditary breast and ovarian cancer (HBOC) syndrome (MedGen UID: 151793) and autosomal recessive Fanconi anemia, type D1 (FA-D1) (MedGen UID: 325420).
- This result is consistent with a predisposition to, or diagnosis of, autosomal dominant BRCA2-related conditions.
- Females with a pathogenic BRCA2 variant have approximately a 40-85% lifetime risk of breast cancer. The risk for contralateral breast cancer 5 years after primary diagnosis is 6.8-9% (PMID: 26239694, 28632866, 25467311). The lifetime risk for ovarian, fallopian tube, or peritoneal cancer is 17-27% (PMID: 9145676, 9497246, 28632866). Males with HBOC have a 7-8% risk for breast cancer (PMID: 20587410) and a 20% risk for prostate cancer (PMID: 10433620). In addition, affected individuals have elevated risks for melanoma and pancreatic cancer (PMID: 10433620).

  Biallelic pathogenic variants in BRCA2 are associated with a particularly severe form of Fanconi anemia (PMID: 16825431) characterized by bone marrow failure, short stature, abnormal skin pigmentation, developmental delay and malformations of the thumbs, skeletal and central nervous systems (PMID: 20417588, 8986277). Risks of leukemia and early onset solid tumors are significantly elevated (PMID: 20507306, 12393424, 12393516), with up to a 97% risk of malignancy by 5 years of age (PMID: 16825431).
- Biological relatives have a chance of being at risk for autosomal dominant BRCA2-related conditions and have a chance of being carriers for autosomal recessive BRCA2-related conditions. Those at risk should consider testing.

Variant details

BRCA2, Intron 20, c.8633-1G>A (Splice acceptor), heterozygous, PATHOGENIC

- This sequence change affects an acceptor splice site in intron 20 of the BRCA2 gene. It is expected to disrupt RNA splicing and likely results in an absent or disrupted protein product.
- This variant is not present in population databases (ExAC no frequency).
- Disruption of this splice site has been observed in individual(s) with increased risk of breast and ovarian cancers (PMID: 16619214, 29446198). ClinVar contains an entry for this variant (Variation ID: 91731).
- Experimental studies have shown that disruption of this splice site disrupts affects mRNA splicing (PMID: 16619214).
- Donor and acceptor splice site variants typically lead to a loss of protein function (PMID: 16199547), and loss-of-function variants in BRCA2 are known to be pathogenic (PMID: 20104584).
- For these reasons, this variant has been classified as Pathogenic.

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Genes analyzed

This table represents a complete list of genes analyzed for this individual, including the relevant gene transcript(s). If more than one transcript is listed for a single gene, variants were reported using the first transcript listed unless otherwise indicated in the report. Results are negative unless otherwise indicated in the report. Benign and Likely Benign variants are not included in this report but are available upon request. An asterisk (*) indicates that this gene has a limitation. Please see the Limitations section for details.

GENE	TRANSCRIPT
ATM	NM_000051.3
ATR	NM_001184.3
BRCA1	NM_007294.3
BRCA2	NM_000059.3
BRIP1	NM_032043.2
CHEK2	NM_007194.3
EPCAM*	NM_002354.2
FANCA	NM_000135.2
GEN1	NM_182625.3
HOXB13	NM_006361.5
MLH1	NM_000249.3
MSH2	NM_000251.2
MSH6	NM_000179.2
NBN	NM_002485.4
PALB2	NM_024675.3
PMS2	NM_000535.5
RAD51C	NM_058216.2
RAD51D	NM_002878.3
TP53	NM_000546.5

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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. Markers across the X and Y chromosomes are analyzed for quality control purposes and may detect deviations from the expected sex chromosome complement. Such deviations may be included in the report in accordance with internal guidelines. 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 Pacific Biosciences (PacBio) SMRT 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 sequenced by PacBio 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). Technical component of Fibroblast cell-culturing and gDNA extraction from skin punch biopsy is performed by Invitae Corporation (5 Technology Drive, Irvine CA 92618, #05D1052995).
- 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), gnomAD (http://gnomad.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/.
- Invitae uses information from individuals undergoing testing to inform variant interpretation. If "Invitae" is cited as a reference in the variant details this may refer to the individual in this requisition and/or historical internal observations.

Limitations

Based on validation study results, this assay achieves >99% analytical sensitivity and specificity for single nucleotide variants, insertions and deletions <15bp in length, and exon-level deletions and duplications. Invitae's methods also detect insertions and deletions larger than 15bp but smaller than a full exon but sensitivity for these may be marginally reduced. Invitae's deletion/duplication analysis determines copy number at a single exon resolution at virtually all targeted exons. However, in rare situations, single-exon copy number events may not be analyzed due to inherent sequence properties or isolated reduction in data quality. Certain types of variants, such as structural rearrangements (e.g. inversions, gene conversion events, translocations, etc.) or variants embedded in sequence with complex architecture (e.g. short tandem repeats or segmental duplications), may not be detected. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity. Unless explicitly guaranteed, sequence changes in the promoter, non-coding exons, and other non-coding regions are not covered by this assay. Please consult the test definition on our website for details regarding regions or types of variants that are covered or excluded for this test. This report reflects the analysis of an

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extracted genomic DNA sample. In very rare cases (such as circulating hematolymphoid neoplasm, bone marrow transplant, recent blood transfusion, or maternal cell contamination), the analyzed DNA may not represent the patient's constitutional genome.

EPCAM: Sequencing analysis is not offered for this gene.

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.

This report has been reviewed and approved by:

Bethany Buckley, Ph.D., FACMG
Clinical Molecular Geneticist