INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

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

Reason for testing

Family history

Test performed

Sequence analysis and deletion/duplication testing of the 84 genes listed in the Genes Analyzed section.

- Invitae Multi-Cancer Panel

RESULT: POSITIVE

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

Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
BRCA2	c.7964A>G (p.Gln2655Arg)	heterozygous	Likely Pathogenic
RECQL4	c.2674G>A (p.Gly892Arg)	heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 84 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.

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Clinical summary

A Likely Pathogenic variant, c.7964A>G (p.Gln2655Arg), 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.

A Variant of Uncertain Significance, c.2674G>A (p.Gly892Arg), was identified in RECQL4.

- The RECQL4 gene is associated with autosomal recessive Rothmund-Thomson syndrome (RTS) (MedGen UID: 10819), RAPADILINO syndrome (MedGen UID: 336602) and Baller-Gerold syndrome (BGS) (MedGen UID: 120532).
- The clinical significance of the identified variant(s) is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

Variant details

BRCA2, Exon 17, c.7964A>G (p.Gln2655Arg), heterozygous, Likely Pathogenic

- This sequence change replaces glutamine with arginine at codon 2655 of the BRCA2 protein (p.Gln2655Arg). The glutamine residue is highly conserved and there is a small physicochemical difference between glutamine and arginine.
- This variant is not present in population databases (ExAC no frequency).
- This variant has been observed in individual(s) with Fanconi anemia (PMID: 20608899). In at least one individual the data is consistent with the variant being in trans (on the opposite chromosome) from a pathogenic variant. ClinVar contains an entry for this variant (Variation ID: 52450).
- Advanced modeling of protein sequence and biophysical properties (such as structural, functional, and spatial information, amino acid conservation, physicochemical variation, residue mobility, and thermodynamic stability) performed at Invitae indicates that this missense variant is expected to disrupt BRCA2 protein function.
- This variant has been reported to affect BRCA2 protein function (PMID:29394989, 29884841).
- In summary, the currently available evidence indicates that the variant is pathogenic, but additional data are needed to prove that conclusively. Therefore, this variant has been classified as Likely Pathogenic.

RECQL4, Exon 15, c.2674G>A (p.Gly892Arg), heterozygous, Uncertain Significance

- This sequence change replaces glycine with arginine at codon 892 of the RECQL4 protein (p.Gly892Arg). The glycine residue is weakly conserved and there is a moderate physicochemical difference between glycine and arginine.
- This variant is not present in population databases (ExAC no frequency).

- This variant has not been reported in the literature in individuals with RECQL4-related conditions.
- Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Tolerated"; PolyPhen-2: "Not Available"; Align-GVGD: "Class C0").
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

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
AIP	NM_003977.3
ALK	NM_004304.4
APC	NM_000038.5
ATM	NM_000051.3
AXIN2	NM_004655.3
BAP1	NM_004656.3
BARD1	NM_000465.3
BLM	NM_000057.3
BMPR1A	NM_004329.2
BRCA1	NM_007294.3
BRCA2	NM_000059.3
BRIP1	NM_032043.2
CASR	NM_000388.3
CDC73	NM_024529.4
CDH1	NM_004360.3
CDK4	NM_000075.3
CDKN1B	NM_004064.4
CDKN1C	NM_000076.2
CDKN2A (p14ARF)	NM_058195.3
CDKN2A (p16INK4a)	NM_000077.4
CEBPA	NM_004364.4
CHEK2	NM_007194.3
CTNNA1	NM_001903.3
DICER1	NM_177438.2
DIS3L2	NM_152383.4
EGFR	NM_005228.3
EPCAM*	NM_002354.2
FH	NM_000143.3
FLCN	NM_144997.5
GATA2	NM_032638.4
GPC3	NM_004484.3
GREM1*	NM_013372.6
HOXB13	NM_006361.5
HRAS	NM_005343.2
KIT	NM_000222.2

GENE	TRANSCRIPT
MAX	NM_002382.4
MEN1	NM_130799.2
MET	NM_001127500.1
MITF*	NM_000248.3
MLH1	NM_000249.3
MSH2	NM_000251.2
MSH3	NM_002439.4
MSH6	NM_000179.2
MUTYH	NM_001128425.1
NBN	NM_002485.4
NF1	NM_000267.3
NF2	NM_000268.3
NTHL1	NM_002528.6
PALB2	NM_024675.3
PDGFRA	NM_006206.4
PHOX2B*	NM_003924.3
PMS2	NM_000535.5
POLD1	NM_002691.3
POLE	NM_006231.3
POT1	NM_015450.2
PRKAR1A	NM_002734.4
PTCH1	NM_000264.3
PTEN	NM_000314.4
RAD50	NM_005732.3
RAD51C	NM_058216.2
RAD51D	NM_002878.3
RB1	NM_000321.2
RECQL4	NM_004260.3
RET	NM_020975.4
RUNX1	NM_001754.4
SDHA*	NM_004168.3
SDHAF2	NM_017841.2
SDHB	NM_003000.2
SDHC	NM_003001.3
SDHD	NM_003002.3

GENE	TRANSCRIPT
SMAD4	NM_005359.5
SMARCA4	NM_001128849.1
SMARCB1	NM_003073.3
SMARCE1	NM_003079.4
STK11	NM_000455.4
SUFU	NM_016169.3
TERC	NR_001566.1
TERT	NM_198253.2
TMEM127	NM_017849.3
TP53	NM_000546.5
TSC1	NM_000368.4
TSC2	NM_000548.3
VHL	NM_000551.3
WRN*	NM_000553.4
WT1	NM_024426.4

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 (310 Goddard, Suite 150, 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 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.

WRN: Deletion/duplication analysis is not offered for exons 10-11. EPCAM: Sequencing analysis is not offered for this gene. GREM1: Promoter region deletion/duplication testing only. MITF: c.952G>A, p.Glu318Lys variant only. SDHA: Deletion/duplication analysis is not offered for this gene. PHOX2B: Alanine repeat numbers for the commonly-expanded region in exon 3 are not determined.

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:

Placeholder for Signature

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INVITAE

GENERAL GUIDELINES

POSITIVE RESULTS GUIDE: BRCA2

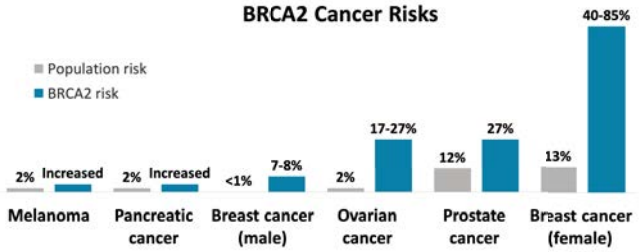
This document is not part of Invitae's clinical report and does not represent medical advice. These are general guidelines that are not specific to your result. You can use this guide to talk to your healthcare provider about your test results, clinical history, and the most current guidelines.

What is a positive BRCA2 result?

A positive test result means that you have a genetic change, called a pathogenic or likely pathogenic variant ("mutation"), in your BRCA2 gene. This variant can cause hereditary breast and ovarian cancer (HBOC) syndrome.

What does this mean?

It's possible for anyone to get cancer in their life, however, people with HBOC are more likely to get breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, and melanoma than the average person. People with one variant in the BRCA2 gene have HBOC. Some people inherit two variants which may cause a rare condition called Fanconi anemia. See the table later in this guide for ways to find and manage HBOC.



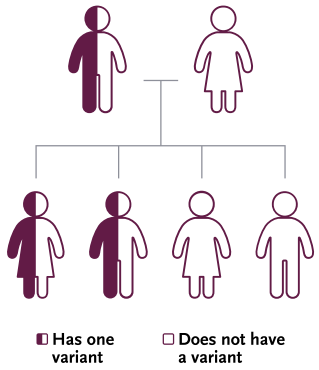
What does this mean for family members?

Genes and variants are passed from generation to generation. Your relatives may also have the same variant(s) in BRCA2. Both men and women can inherit and pass on this type of variant.

Who should be tested next?
Your close relatives have a chance of also having the same positive variant. This means your parents, siblings, and children. Your other relatives may also have the same BRCA2 variant(s). People with variants in BRCA2 have different conditions or symptoms depending on whether they inherit one or two variants.

Inheriting this BRCA2 variant does not mean that a person will definitely develop cancer. A variant in the BRCA2 gene affects everyone differently. Family members may develop the condition at different ages, or they may develop different features. Features of this condition usually do not affect children. Genetic testing for this variant is not recommended until the age of 18.

Chance for passing on a variant



Genetic testing is a personal choice and your family members may choose not to have genetic testing. It is recommended that they talk with their own healthcare provider about a plan for screening.

Create a plan with your healthcare provider

These options are a guide for you and your healthcare provider. They are meant to be used along with your genetic test results and other health information. Each option may or may not be right for you. Your positive test result on its own can not predict how this condition may affect you. Please talk with your healthcare provider to make a plan that's right for you.

Options you and your healthcare provider might consider

CONDITION	RISK FOR GENERAL POPULATION	RISK FOR BRCA2	OPTION	MORE INFORMATION
Breast cancer (female)	13%	40-85%	• Periodic breast self exam starting at age 18 (1)	• Helps find cancer so you can seek treatment as soon as possible.
			• Clinical breast exam every 6-12 months, starting at age 25 (1)	• Helps find cancer so you can seek treatment as soon as possible. • If you have a family history of breast cancer, screening may be started earlier than age 25.
			• Breast MRI with contrast once per year from ages 25–29 (1)	• Helps find cancer so you can seek treatment as soon as possible. • If a breast MRI is not available, consider a mammogram or a 3D mammogram. • If you have a family history of breast cancer, screening may be started earlier than age 25.
			• Mammogram or 3D mammogram and a breast MRI with contrast once per year from ages 30-75 (1)	• Helps find cancer so you can seek treatment as soon as possible. • If you have a family history of breast cancer, screening may be started earlier than age 30. • Your personal and family health history will help determine if this screening is appropriate over age 75
			• Consider risk-reducing mastectomy (surgery to remove the breasts) based on personal and family health history (1)	• Can help prevent cancer.
Breast cancer (male)	<1%	7-8%	• Breast self exam starting at age 35 (1)	• Helps find cancer so you can seek treatment as soon as possible.
			• Clinical breast exam once per year starting at age 35 (1)	• Helps find cancer so you can seek treatment as soon as possible. • If you have a family history of breast cancer, screening may be started earlier than age 35.
Ovarian cancer	2%	17-27%	• Consider transvaginal ultrasound with CA-125 blood testing once per year starting at age 35 for those that do not yet have their ovaries removed (1)	• Helps find cancer so you can seek treatment as soon as possible. • The benefit of these tests is unknown.
			• Consider risk-reducing salpingo-oophorectomy (RRSO) (surgery to remove the ovaries and fallopian tubes) from ages 35-40 but may be delayed to 40-45 for those with BRCA2 risk, after child bearing is complete (1)	• Helps prevent cancer. • Your personal and family health history will help determine if and when to consider this option.
Prostate cancer	12%	27%	• Prostate specific antigen (PSA) blood screening starting at age 40 (1)	• Helps find cancer so you can seek treatment as soon as possible.
Pancreatic cancer	2%	Increased	• Consider pancreatic imaging (MRI with contrast or endoscopic ultrasound) if there is additional family history of pancreatic cancer (1)	• Helps find cancer so you can seek treatment as soon as possible. • Results of this test will help determine if and when you will need more testing.

CONDITION	RISK FOR GENERAL POPULATION	RISK FOR BRCA2	OPTION	MORE INFORMATION
Melanoma	2%	Increased	• Full body skin examination (1)	• Helps to find cancer so you can seek treatment as soon as possible. • This represents general melanoma risk management, since no additional screening guidelines have been established for people with a positive BRCA2 variant.
			• Minimize UV exposure (1)	

These options outline recommendations from NCCN. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Guideline Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version V.1.2020. (1) © National Comprehensive Cancer Network, Inc. 2019. All rights reserved. Accessed December 19, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. We are always learning more about genetics and disease, so please always refer to the current guidelines and recommendations when considering surveillance and treatment options. Information in this document may not include all relevant international recommendations and acts as a supplement to the Invitae result report. This information is not meant to replace a discussion with your healthcare provider and should not be considered or interpreted as medical advice.

We (and others) are here to help

Genetic counseling is recommended to help you clearly and accurately understand your results so it's important to talk to your genetic counselor or other healthcare provider about your test results. Invitae also has board-certified genetic counselors who are available to answer questions about your test results or these options. Log in to your patient portal (invitae.com) to view your results, search for a local or Invitae genetic counselor, or join Invitae's Patient Insight Network (PIN), a community where you can connect with other patients and share your experience.

Notes for personalized assessment