INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

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

Reason for testing
Diagnostic test for a personal and family history of disease

Test performed
Sequence analysis and deletion/duplication testing of the 83 genes listed in the results section below.

- Invitae Multi-Cancer Panel

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.
Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
BRCA2	c.4638del (p.Phe1546Leufs*22)	heterozygous	PATHOGENIC
PALB2	c.2482T>C (p.Cys828Arg)	heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 83 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.
- 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 Pathogenic variant, c.4638del (p.Phe1546Leufs*22), 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.2482T>C (p.Cys828Arg), was identified in PALB2.

- The PALB2 gene is associated with autosomal dominant predisposition to breast, pancreatic and possibly ovarian cancer (PMID: 25099575, 17200668, 18628482) and autosomal recessive Fanconi anemia (MedGen UID: 372133).
- The clinical significance of this result 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/en/family/.

Variant Details

BRCA2, Exon 11, c.4638del (p.Phe1546Leufs*22), heterozygous, PATHOGENIC

- This sequence change creates a premature translational stop signal (p.Phe1546Leufs*22) in the BRCA2 gene. It is expected to result in an absent or disrupted protein product.
- This variant is not present in population databases (ExAC no frequency).
- This variant has been observed in individuals affected with breast and ovarian cancer (PMID: 11044354, 14647210, 15131399, 17148771, 21324516, 26296701). This variant is also known as 4862delT and 4866delT in the literature. ClinVar contains an entry for this variant (Variation ID: 37915).
- Loss-of-function variants in BRCA2 are known to be pathogenic (PMID: 20104584).

- For these reasons, this variant has been classified as Pathogenic.

PALB2, Exon 5, c.2482T>C (p.Cys828Arg), heterozygous, Uncertain Significance

- This sequence change replaces cysteine with arginine at codon 828 of the PALB2 protein (p.Cys828Arg). The cysteine residue is moderately conserved and there is a large physicochemical difference between cysteine and arginine.

- This variant is not present in population databases (ExAC no frequency) and has not been reported in the literature in individuals with a PALB2-related disease. ClinVar contains an entry for this variant (Variation ID: 233646).

- Algorithms developed to predict the effect of missense changes on protein structure and function output the following: (SIFT: "Tolerated"; PolyPhen-2: "Benign"; Align-GVGD: "Class C0"). The arginine amino acid residue is found in multiple mammalian species, suggesting that this missense change does not adversely affect protein function. These predictions have not been confirmed by published functional studies.

- In summary, this variant is a rare missense change that is not predicted to affect protein function. There is no indication that it causes disease, but the available evidence is currently insufficient to prove that conclusively. Therefore, it has been classified as a Variant of Uncertain Significance.

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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. 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
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
MAX	NM_002382.4
MEN1	NM_130799.2

GENE	TRANSCRIPT
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
SMAD4	NM_005359.5
SMARCA4	NM_001128849.1
SMARCB1	NM_003073.3

GENE	TRANSCRIPT
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

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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. 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).

- The following transcripts were used in this analysis. 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: 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), 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), 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).

- 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/.

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- 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, (circulating hematolymphoid neoplasm, bone marrow transplant, recent blood transfusion) the analyzed DNA may not represent the patient's constitutional genome. GREM1: Promoter region deletion/duplication testing only. EPCAM: Deletion/duplication testing only (NM_002354.2). MITF: c.952G>A, p.Glu318Lys variant only. NTHL1: Deletion/duplication analysis is not offered for this gene (NM_002528.6). PHOX2B: Alanine repeat numbers for the commonly-expanded region in exon 3 are not determined. WRN: Deletion/duplication analysis is not offered for exons 10 or 11 (NM_000553.4). EGFR: c.2369C>T (p.Thr790Met), c.2327G>A (p.Arg776His), c.2527G>A (p.Val843Ile) variants only. HOXB13: c.251G>A, p.Gly84Glu variant only.

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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