PREVENTION GENETICS part of EXACT SCIENCES

NAME	PATIENT ID
TEST, test	2022-243-612

riskguard™

Hereditary Cancer Test

DEMOGRAPHICS

DOE, Jane ID#: 123-456-789 DOB: January 01, 1950 Sex: Female	Type: Whole Blood Collected: August 20, 2022 Received: August 29, 2022 PG ID: 2022-243-612	Provider Genetic Counselor Institution

SUMMARY FINDINGS

POSITIVE

RESULTS

Sequence Variant(s):

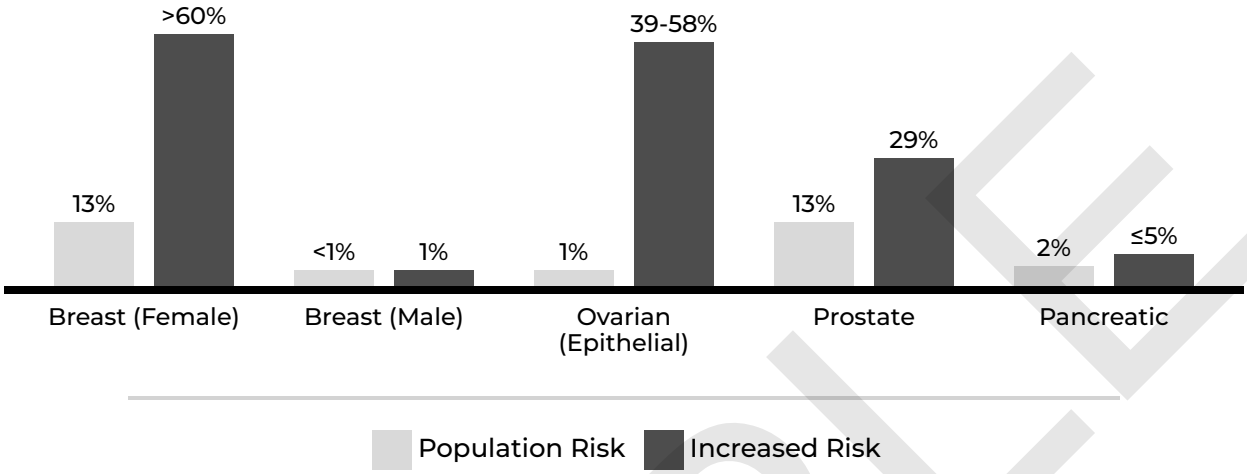
Gene	Variant Zygosity	Classification
BRCA1	c.66dup, p.Glu23Argfs*18, Heterozygous	PATHOGENIC

CARE POINTS

- A positive result in the *BRCA1* gene is associated with hereditary breast and ovarian cancer syndrome (HBOC). People with HBOC are at increased risk for breast, ovarian, pancreatic, prostate and possibly other cancers.
- People who inherit two pathogenic variants in *BRCA1* can develop Fanconi anemia, a childhood-onset disorder causing physical abnormalities, bone marrow failure and increased cancer risk.
- The individual is encouraged to share these results with family members who may be at risk of having the same genetic variant.

- This individual should work with their healthcare providers to develop a tailored management plan. See care options on following page for general guidelines. These are not meant to be comprehensive or specific to any individual patient.
- Genetic counseling is recommended.

LIFETIME CANCER RISK
Associated with *BRCA1* Variants



*Some patients may have an increased lifetime cancer risk based on other patient specific risk factors.

CARE OPTIONS

SURVEILLANCE

Breast Cancer

Breast self exam	Monthly
Clinical breast exam	Every 6 months beginning at age 25
Breast MRI	Annually beginning at age 25
Mammogram	Annually beginning at age 30

Ovarian Cancer

Transvaginal ultrasound	Annually beginning at age 30
CA-125	Annually beginning at age 30

THERAPEUTIC

Breast Cancer

Risk-reducing medication	Individualized
Risk-reducing mastectomy	Individualized

Ovarian Cancer

Risk-reducing salpingo-oophorectomy	Individualized
Risk-reducing medication	Individualized

SURVEILLANCE

THERAPEUTIC

Endometrial

Hysterectomy	Individualized

Breast Cancer (Male)

Clinical breast exam	Annually beginning at age 35

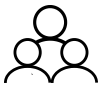
Prostate Cancer

Prostate-specific antigen (PSA)	Annually beginning at age 40
Digital rectal exam	Annually beginning at age 40

Pancreatic Cancer

Endoscopic ultrasound and/or MRI/Magnetic resonance cholangiopancreatography	Individualized

FAMILIAL RISK



1. Family members who have the variant may benefit from early screening or preventive care.

2. Variants in the *BRCA1* gene are usually inherited from one parent. Parents, siblings, and children have a 1 in 2 (50%) chance of having the same variant. Other family members (such as grandparents, aunts/uncles, and cousins) on the same side of the family may also have the variant.

1. Family members should talk to their healthcare provider if they are interested in genetic testing.

CANCER SEQUENCING PANEL with CNV DETECTION

Gene, Transcript	Mode of Inheritance, Gene OMIM	DNA Variants, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
BRCA1, NM_007294.3	AD, AR, 113705	c.66dup, p.Glu23Argfs*18, Heterozygous	37691	Not Present	Not Applicable	PATHOGENIC

Mode of Inheritance: Autosomal Dominant=AD, Autosomal Recessive=AR, X-Linked=XL

ClinVar ID: Variant accession (www.ncbi.nlm.nih.gov/clinvar)

Allele Frequency registered in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported within one of seven population categories recognized in gnomAD v.2.0 (The "Other" population is excluded).

Missense Predictions: Summarized output (Damaging, Conflicting, or Tolerated) via PolyPhen-2, SIFT, MutationTaster, and FATHMM (PMID: 26555599).

RESULTS and INTERPRETATIONS

BRCA1 VARIANT INFORMATION

This patient is heterozygous in the *BRCA1* gene for a sequence variant defined as c.66dup, which is predicted to result in a frameshift and premature protein termination (p.Glu23Argfs*18). This variant is alternatively referred to as 185insA. This variant has been reported in many individuals and families with breast and/or ovarian cancer (Matsushima et al. 1995. PubMed ID: 8595420; Couch et al. 1996. PubMed ID: 8807330; Liede et al. 2002. PubMed ID: 12181777; Rashid et al. 2006. PubMed ID: 16998791; Noël et al. 2010. PubMed ID: 20189727; PubMed ID: 24916970; Arai et al. 2017. PubMed ID: 29176636; Heramb et al. 2018. PubMed ID: 29339979; Li et al. 2018. PubMed ID: 29752822; Bhaskaran et al. 2019. PubMed ID: 30702160). It has also been reported in a male individual with melanoma (Ibrahim et al. 2018. PubMed ID: 29433453). This variant has not been reported in a large population database (http://gnomad.broadinstitute.org), indicating this variant is rare. In ClinVar, this variant is interpreted as pathogenic (https://www.ncbi.nlm.nih.gov/clinvar/variation/37691/). Frameshift variants in *BRCA1* are expected to be pathogenic. This variant is interpreted as pathogenic.

Pathogenic variants in *BRCA1* have been associated with autosomal dominant familial breast-ovarian cancer 1 (OMIM #604370), autosomal dominant susceptibility to pancreatic cancer 4 (OMIM #614320), and autosomal recessive Fanconi anemia, complementation group S (OMIM #617883).

This patient is apparently negative for copy number variants (CNVs) within the genomic regions of this test.

These results should be interpreted in the context of clinical findings, family history and other laboratory data.

All genetic tests have limitations. See limitations and other information for this test on the following page(s).

GENES ANALYZED

APC, *ATM*, *AXIN2*, *BARD1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EPCAM*, *GREM1*, *HOXB13*, *MLH1*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NBN*, *NTHL1*, *PALB2*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RAD51C*,

RAD51D, *SMAD4*, *STK11*, *TP53*

SUMMARY STATISTICS

Pipeline	Version	Average NGS Coverage	Fraction Bases Covered with NGS
Infinity_Pipeline	1.8.12	279x	100.0%

Minimum NGS coverage is ≥20x for all coding exons and +/-10bp of flanking DNA.

Electronically signed on September 14, 2022 by:
Gregory Fischer, PhD
Human Molecular Geneticist

Electronically signed and reported on September 14, 2022 by:
Diane Allingham-Hawkins, PhD, FCCMG, FACMG
Laboratory Director

SUPPLEMENTARY INFORMATION and TEST METHODS

The 32-gene Riskguard™ Hereditary Cancer Test includes germline DNA-based Next Generation Sequencing (NGS) and Copy Number Variation (CNV) analysis. Sequence and CNV analysis are performed for the following genes: *APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11,* and *TP53*. Only CNV analysis is reported for *EPCAM*. The panel includes testing of *APC* promoter 1B, the inversion of exons 1 to 7 in *MSH2* (Boland Inversion), and the *MSH2* c.942+3A>T polyalanine repeat variant.

Patient samples are assigned a unique barcode for sample tracking. Genomic DNA is extracted and purified from whole blood (K2EDTA), buccal (OCD-100 swab), saliva (Oragene™ or GeneFiX™), cell culture, or fresh tissue for molecular testing. Extracted genomic DNA is also an acceptable sample type for molecular testing. As required, genomic DNA is extracted from the sample. The DNA corresponding to these regions is captured using hybridization probes. Captured DNA is sequenced using Illumina's Reversible Dye Terminator (RDT) platform NovaSeq 6000 (Illumina, San Diego, CA, USA). The resulting reads are then filtered by quality and aligned to a reference sequence for analysis. Once sequencing is complete, bioinformatic analysis is triggered. The PreventionGenetics analysis pipeline utilizes a mix of vendor and custom software for NGS data processing.

Multi-gene panel testing via NGS analysis provides approximately 99.8% coverage of all coding exons of the panel genes plus 10 bases of flanking noncoding DNA in all available transcripts along with other non-coding regions in which pathogenic variants have been identified at PreventionGenetics or reported elsewhere. Coverage is defined as ≥20X NGS reads or Sanger sequencing.

For Sanger sequencing, Polymerase Chain Reaction (PCR) is used to amplify the necessary exons plus additional flanking non-coding sequence. After purification of the PCR products, cycle sequencing is conducted using the Applied Biosystems Incorporated (ABI) Big Dye Terminator v.3.1 kit. PCR products are resolved by electrophoresis on an ABI 3730xl capillary sequencer. In most cases, cycle sequencing is performed separately in both the forward and reverse directions; in some cases, sequencing is performed twice in either the forward or reverse directions. In some instances, allele-specific PCR may also be utilized.

Unless present within coding regions, runs of mononucleotide repeats (e.g. (A)n or (T)n) with n >8 in the reference sequence) are generally not analyzed because of strand slippage during amplification. As such, this test does not include analysis of the polyalanine repeat in *MSH3* (NM_002439:c.151 to c.380).

Deletion and duplication testing for *STK11* and *MS2* is performed using NGS, but CNVs detected in these genes are confirmed via multiplex ligation-dependent probe amplification (MLPA). Where necessary, nested long-range PCR and Sanger sequencing is performed for clinically actionable variants within *PMS2* (NM_000535) exons 11 through 15, which share homology with the pseudogene *PMS2CL*.

The ability to detect retrotransposon elements such as the *BRCA2* Portuguese founder variant NM_000059:c.156_157insAlu (also known as 384insAlu) may not be technically possible due to location and complexity. In these instances, the retrotransposon element will not be included on the report.

GREM1 analysis does not currently include analysis of the upstream gene *SCG5*.

Detection of minor sequence variants due to somatic mosaicism is limited. Sequence variants that are present in less than 15% of the patient's nucleated cells may not be detected.

CNVs are detected from NGS data using a CNV calling algorithm that compares mean read depth and distribution for each target in the test sample against multiple matched controls. Neighboring target read depth and distribution and zygosity of any variants within each target region are used to reinforce CNV calls. If Quality Control (QC) metrics are not met, independent (orthogonal) confirmation of clinically relevant CNVs may be performed using alternative methodologies including MLPA, Chromosomal Microarray Analysis (CMA), gene-centric array Comparative Genomic Hybridization (aCGH), or long-range PCR analysis and/or sequencing of the resulting PCR product. On occasion, it will not be technically possible to confirm a smaller CNV called by NGS. In these instances, the CNV will not be included on the report.

Indication for Use and Intended Patient Population

The Riskguard Hereditary Cancer Test is intended for the identification of hereditary risk associated with eight common types of cancer: breast, ovarian, prostate, endometrial, colorectal, pancreatic, gastric, and melanoma. Additional types of cancer, polyposis predisposition, and cancer related syndromes are also included. Of note, several genes on this panel are associated with rare autosomal recessive cancer-related syndromes that may pose a risk to offspring if an individual's partner is also a carrier. The majority of genes included on this panel are clinically actionable with medical society guidelines available on risk management for carriers of pathogenic germline variants. This test is intended for individuals with a clinical indication for germline testing for hereditary cancer. This test is specifically designed for heritable germline variants and is not appropriate for the detection of somatic variants in tumor tissue.

Current Clinical Guidelines

Testing utilizes the Feb. 2009 assembly of the human reference genome (hg19, GRCh37 Genomic Reference Consortium Human Reference 37 [GCA_000001405.1]). All differences from the reference sequence (sequence variants) are assigned to one of six interpretation categories: Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign, Benign (per Richards et al. 2015. PubMed ID:25741868), and Risk allele per laboratory established classifications. Risk alleles may not be listed on the report but are available upon request. Benign and Likely Benign variants are not listed in the reports but are available upon request.

Human Genome Variation Society (HGVS) nomenclature recommendations are used to describe sequence variants (https://www.hgvs.org). The International System for Human Cytogenomic Nomenclature (ISCN) and HGVS nomenclature (if applicable) are used to describe CNVs (https://iscn.karger.com/; https://www.hgvs.org).

REGULATORY INFORMATION

These results should be used in the context of available clinical findings and should not be used as the sole basis for treatment. This test was developed, and its performance characteristics determined by PreventionGenetics. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

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