Patient:
DOE, JANE

Accession: NXGMDX-XXXXXX
Created: August 18, 2021
Patient Gender: Female
Date Of Birth: January 01, 19XX
Specimen Type: Whole blood
Collection Date: August 18, 2021

Receiving Facilities:
SAMPLE REPORT

Receiving Physicians:
Dr. Smith

BRCA PANEL RESULTS SUMMARY

POSITIVE: A Pathogenic Variant Was Identified

Gene	Result	Result is associated with the following cancer risks:
BRCA2	c.8754+4A>G (None) Zygosity: Heterozygous	**HIGH RISK: Female Breast, Ovarian, Male Breast** **ELEVATED RISK: Pancreatic, Melanoma, Prostate**

Interpretation

•This result is consistent with a diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome.
• Risk estimate: >60% lifetime risk of female breast cancer; 13-29% lifetime risk of ovarian cancer, >6% lifetime risk for male breast cancer, 15% lifetime risk for prostate cancer, 5-10% lifetime risk for pancreatic cancer and an increased lifetime risk for melanoma (NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian. V 2.2022).
• This result does not mean the individual has a diagnosis of cancer or that they will definitely develop cancer in their lifetime.
• Genetic testing for pathogenic variants in family members can help identify at-risk individuals.
• Genetic counseling is recommended for all individuals undergoing genetic testing.

Genes Tested: *BRCA1, BRCA2*

Supporting Evidence

This patient is heterozygous for variant c.8754+4A>G associated with the *BRCA2* gene. Inherited mutations in *BRCA1* and this gene, *BRCA2*, confer increased lifetime risk of developing breast or ovarian cancer. Both *BRCA1* and *BRCA2* are involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. The *BRCA2* protein contains several copies of a 70 aa motif called the BRC motif, and these motifs mediate binding to the *RAD51* recombinase which functions in DNA repair. *BRCA2* is considered a tumor suppressor gene, as tumors with *BRCA2* mutations generally exhibit loss of heterozygosity (LOH) of the wild-type allele. [provided by RefSeq, Dec 2008]

BRCA2 (NM_007294.4):c.8754+4A>G (splicing), Likely Pathogenic
This *BRCA2* variant is denoted c.8754+4A>G at the cDNA level and disrupts splicing of the *BRCA2* gene. This variant is not present in population databases (ExAC no frequency) and has been reported in the literature in individuals with breast, ovarian, and other cancers (PMID:20927582, 23096105). Functional studies have shown that this variant causes loss of the native donor site and use of a cryptic splice site, resulting in premature truncation (PMID:17011978, 22505045, 25382762). Based on currently available evidence, we consider *BRCA2* c.8754+4A>G a likely pathogenic variant.

JANE DOE's Report

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Methods

The NxGen MDx Hereditary Cancer Test is a comprehensive screen of 32 genes associated with hereditary cancer predisposition. DNA is isolated from the patient's specimen using standardized methodology and quantified. Targeted regions of the genes listed in the disease table are amplified enzymatically and subjected to next generation sequencing (NGS) on Ion Torrent sequencing platform in the NxGen MDx laboratory. Targeted regions are sequenced with ≥50x average read depth. Enrichment and analysis focus on the coding sequence and untranslated regions of the indicated transcripts, ≥20bp of flanking intronic sequence, and other regions known to be relevant to hereditary cancer at the time of assay design. The DNA sequences are assembled and aligned against reference gene sequences based on the human genome build GRCh37/UCSC hg 19 and analyzed for sequence variants using software from ThermoFisher, as well as proprietary software developed by the NxGen MDx bioinformatics team. Our median coverage across our samples is >250X (can exceed 1000X) and our minimum acceptance criteria for depth is: >98% at 20X

Some variants may not be detected in areas of low sequence coverage. Mosaicism or somatic variants present at low levels may not be detected. Suspect variant calls other than those classified as "likely benign," "benign," or "VUS" are verified by Sanger sequencing. Gross deletion/duplication analysis is performed using the Ion Reporter software with confirmatory multiplex ligation-dependent probe amplification (MLPA).

Sequence variants are analyzed using ACMG-AMP variant interpretation guidelines in conjunction with a variety of resources including ClinVar, robust review of the literature for functional studies, and in vivo models, population allele frequency, and in silico predictive tools. This test targets detection of pathogenic variants in 32 genes (APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, FANCC, GREM1, MLH1, MSH2, MSH6, MRE11, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, SMARCA4, STK11, and TP53). In addition, sequencing of the promoter region is performed for the following genes: PTEN (c.-1300 to c.-745), MLH1 (c.-337 to c.-194), and MSH2 (c.-318 to c.-65). Variants of unknown significance detected outside of the coding region are not routinely reported. For POLD1 and POLE, missense variants located outside of the exonuclease domains (codons 311-541 and 269-485, respectively) are not routinely reported. The BRCA2 Portuguese founder variant, c.156_157insAlu (also known as 384insAlu), and the MSH2 coding exons 1-7 inversion are detected by next generation sequencing and confirmed by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA), respectively. For GREM1, only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For EPCAM, only gross deletions encompassing the 3' end of the gene are reported. The APC promoter 1B region is covered as part of deletion/duplication analysis. If a deletion is detected in exons 13, 14, or 15 of PMS2, long-range PCR (LR-PCR) is used to isolate the PMS2 gene exons 12-15 and c-terminal like pseudogene, PMS2CL, and the LR-PCR products are Sanger sequenced to verify that the variant is present in the gene of interest, PMS2, and not the pseudogene.

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Disclaimer

This test was developed and its performance determined and validated by NxGen MDx. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA), however, the FDA has determined that such a clearance or approval is not necessary. The 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.

A negative result cannot rule out the possibility that the tested individual carries rare pathogenic variant(s) not reported in the literature. The test is designed and validated to detect ~99% of described pathogenic variants in the 32 genes represented on the panel (analytical sensitivity). The clinical sensitivity of the test may vary widely according to the specific clinical and family history. Breast, ovarian, and colon cancers are complex clinical disorders. Pathogenic variants in other genes or the regions not analyzed by the test can also give rise to clinical conditions similar to breast, ovarian, colon, or other cancer.

Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that interfere with analysis, low level mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, presence of premalignant or malignant cells in the sample, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature.

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HEREDITARY CANCER GENES ANALYZED

NxGen MDx

Genes	Breast	Ovarian	Uterine	Colorectal	Gastric	Pancreatic	Prostate	Melanoma
APC				●	●	●		
ATM	●					●	●	
BARD1	●							
BMPR1A				●	●	●		
BRCA1	●	●				●	●	
BRCA2	●	●				●	●	●
BRIP1	●	●						
CDH1	●			●	●			
CDK4						●		●
CDKN2A						●		●
CHEK2	●			●			●	
DICER1		●						
EPCAM*		●	●	●	●	●	●	
FANCC	●					●		
GREM1*				●				
MLH1		●	●	●	●	●	●	
MSH2		●	●	●	●	●	●	
MSH6		●	●	●	●	●	●	
MRE11	●	●						
MUTYH				●				
NBN	●						●	
PALB2	●					●	●	
PMS2		●	●	●	●	●		
POLD1				●				
POLE				●				
PTEN	●		●	●				●
RAD51C		●						
RAD51D		●						
SMAD4				●	●	●		
SMARCA4		●						
STK11	●	●	●	●	●	●		
TP53	●	●	●	●	●	●	●	●

*Deletion/ Duplication Only

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Management Information for *BRCA2*

This overview of clinical management guidelines is based on this patient's positive test result for a *BRCA2* gene variant. Unless otherwise stated, medical management guidelines used here are limited to those issued by the National Comprehensive Cancer Network® (NCCN®)[1] in the U.S. Please consult the referenced guideline for complete details and further information.

Clinical correlation with the patient's past medical history, treatments, surgeries, and family history may lead to changes in clinical management decisions; therefore, other management recommendations may be considered. Genetic testing results and medical society guidelines help inform medical management decisions but do not constitute formal recommendations. Discussions of medical management decisions and individualized treatment plans should be made in consultation between each patient and their healthcare provider and may change over time.

Screening/Surgical Considerations	Age to Start	Frequency
Female Breast Cancer[1]		
Breast awareness • Women should be familiar with their breasts and promptly report changes to their healthcare provider.	18 years old	Periodic and consistent
Clinical breast exam	25 years old	Every 6-12 months
Breast Screening • Mammography with consideration of tomosynthesis • Consider breast MRI with contrast	25-29 years old: MRI or mammogram (if MRI unavailable) 30-75 years old: MRI and mammogram >75 years old: individualized management	Every 12 months
Discuss option of risk-reducing mastectomy	Individualized	N/A
Consider investigational imaging and screening studies, when available in context of a clinical trial	Individualized	Individualized
Consider options for risk reduction agents, such as chemoprevention (i.e. tamoxifen)	Individualized	Individualized
Ovarian Cancer[1]		
Recommend risk-reducing salpingo-oophorectomy (RRSO)	35 to 40 years old, upon completion of child bearing	N/A
If RRSO not elected, transvaginal ultrasound and serum CA-125 have not been shown to be sufficiently sensitive or specific as to support a positive recommendation, but may be considered	30-35 years old	Clinician's discretion

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Consider investigational imaging and screening studies, when available in the context of a clinical trial (i.e. salpingectomy alone)	Individualized	Individualized
Consider options for risk reduction agents, such as chemoprevention (i.e. oral contraceptives)	Individualized	Individualized

Male Breast Cancer[1]

Breast self-exam training and education	35 years old	Periodic and consistent
Clinical breast exam	35 years old	Every 12 months

Prostate Cancer[1]

Recommend prostate cancer screening	45 years old	Clinician's discretion

Pancreatic Cancer[1]

No specific screening guidelines exist, but may be considered based on cancers seen in the family	Individualized	N/A

Screening of Other General Population Cancer Risks

Colon Cancer[2]

Colonoscopy	45 years old or 10 years younger than the earliest diagnosis in the family	Every 10 years

Skin Cancer[3]

Limit exposure to UV light, wear a hat, sunglasses, protective clothing, and apply sunscreen with SPF 30 or higher	Childhood	N/A

General Recommendations For All Individuals

Family Planning

- Individuals of reproductive age who are concerned about the possibility of passing on a *BRCA2* variant to a future child may want to discuss options for prenatal testing and assisted reproduction techniques, such as pre-implantation genetic diagnosis (PGD).
- Individuals with *BRCA2* variants may have an increased risk to have a child with Fanconi anemia, but only if their partner also carries a variant in the *BRCA2* gene. Fanconi anemia is a rare condition that can cause specific physical characteristics, bone marrow failure, and an increased risk of certain cancers.

Lifestyle and Environmental Exposures

- Avoid all forms of tobacco
- Get to and stay at a healthy weight
- Get moving with regular physical activity
- Eat healthy with plenty of fruits and vegetables
- Limit how much alcohol you drink (if you drink at all)
- Know yourself, your family history, and your risks
- Get regular check-ups and cancer screening. A cancer-related check-up should include health counseling and, depending on a person's age and gender, exams for cancers of the thyroid, oral cavity, skin, lymph nodes, testes, and ovaries, as well as for some other diseases besides cancer.

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Resources for *BRCA2*

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 NxGen MDx genetic counselor please call 855-776-9436, ext. 1. If you would like to find a local genetic counselor, visit www.nsgc.org or tagc.med.sc.edu/professional_organizations.asp.

Please discuss this inormation with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your *BRCA2* result, medical recommendations, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider and should not be considered or interpreted as medical advice.

Resources and Organizations

- Bright Pink brightpink.org
- FORCE facingourrisk.org
- Sharsheret sharsheret.org
- Susan G. Komen Foundation komen.org
- Genetic Information Nondiscrimination Act (GINA) ginahelp.org
- National Society of Genetic Counselors nsgc.org
- Canadian Society of Genetic Counsellors cagc-accg.ca

1. NCCN Clinical Practice Guidelines in Oncology®. Genetic/Familial High-Risk Assessment: Breast and Ovarian. V3.2019. Available at www.nccn.org.
2. NCCN Clinical Practice Guidelines in Oncology®: Colon Cancer Screening . V1.2018. Available at www.nccn.org
3. NCCN Clinical Practice Guidelines in Oncology®: Melanoma. V 1.2018 Available at www.nccn.org

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Opportunity to Enroll in Hereditary Cancer Research

Genetic testing can help individuals and families by giving them a clear idea of their cancer risks. Genetic tests (called multi-gene or multiplex panels) look for changes in several genes, all in a single test. While all of the genes on these panels have been tied to an increased risk of cancer, we understand the risks associated with some of the genes better than we understand others. One way to help improve our understanding is to enroll people with pathogenic variants or variants of unknown significance in registries. Registries typically follow people over many years to learn more about these alterations and how they impact their health.

How can I find a research registry?

There are several hereditary cancer research registries that are studying individuals who have had multi-gene panel testing. One registry that is open to individuals nationwide is the Prospective Registry of MultiPlex Testing (PROMPT). PROMPT is a joint effort involving several academic medical centers and commercial laboratories, working together to learn more about the genes that are studied on multi-gene panels. PROMPT will allow researchers to better understand the cancer risks associated with changes in these genes and thus provide a better understanding of the best way to take care of individuals who have such changes.

What is involved in participation?

Participation in the study simply involves completing online surveys. You can take part without providing any personal information to the PROMPT study. If you are interested, the PROMPT team will reach out to you to talk about ways that you can get more involved with the research effort. Either way, your participation will help researchers learn more and improve the ability of this genetic testing to help people.

How do I enroll?

You can learn more about or register for PROMPT by going to www.promptstudy.org or by scanning the QR code to the right.

Thank you again for considering taking part in PROMPT!

If you would like to read more about multi-gene panels, including details about specific genes, please visit the informational website at www.promptstudy.info.

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