INVITAE

Name	DOB
Jane Doe	

Patient Name	DOB	Sex	MRN	Invitae #
Jane Doe				

Clinical Team	Report Date	Sample Type	Sample Collection Date	Sample Accession Date

Test Performed
Sequence analysis and deletion/duplication testing of the 42 genes listed in the results section below.
- Invitae Common Hereditary Cancers Panel (Breast, Gyn, GI)

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

Summary

Positive result. Pathogenic variant identified in ATM.
Variant of Uncertain Significance identified in BARD1.

Clinical Summary

- A Pathogenic variant, c.2250G>A (Silent), was identified in ATM.
  - The ATM gene is associated with an increased risk for autosomal dominant breast and pancreatic cancer (PMID: 15928302, 15942625, 16998505, 22585167, 26483394, 26662178). There is also preliminary evidence supporting a correlation with autosomal dominant colorectal, prostate, and possibly other cancers (PMID: 15928302, 15942625, 26662178). Additionally, the ATM gene is associated with autosomal recessive ataxia-telangiectasia (A-T) (MedGen UID: 439).
  - This result is consistent with a predisposition to, or diagnosis of, autosomal dominant ATM-related conditions.
  - The lifetime risk of breast cancer in females with one pathogenic ATM variant is 17-52% (PMID: 3574400, 15928302, 16832357, 16958054). The risk of developing pancreatic cancer is also elevated (PMID: 22585167, 26098866, 26483394). The risk of other cancers such as colorectal and prostate cancers may be increased as well, although available evidence is not sufficient to make a determination at this time (PMID: 15928302, 15942625, 26098866, 26662178). Clinical cancer management guidelines for ATM can be found at www.nccn.org.
  - Close relatives (children, siblings, and each parent) have up to a 50% chance of being a carrier of this variant. More distant relatives may also be carriers. Carriers are at increased risk of developing autosomal dominant ATM-related conditions and may have reproductive risks related to autosomal recessive ATM-related conditions as well. Testing for this variant is available.
- A Variant of Uncertain Significance, c.944C>T (p.Pro315Leu), was identified in BARD1.
  - The BARD1 gene is associated with an increased risk for autosomal dominant breast and possibly ovarian cancer in individuals who carry a single pathogenic BARD1 variant (MedGen UID: 87542) (PMID: 21344236, 20077502, 22006311, 16825437).
  - The clinical significance of this variant is uncertain at this time. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
  - This variant is not eligible for complimentary family studies as part of our VUS Resolution Program because the results are unlikely to assist Invitae in reclassifying this particular variant. However, if desired, testing for this variant in other family members can be ordered at a reduced cost through the Family Variant Testing Program. Details on our VUS Resolution and Family Variant Testing Programs can be found at www.invitae.com.

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- Previous analysis performed at a different laboratory reportedly identified a variant in MSH2, IVS4+5 A>G, currently known as c.792+5A>G (Intronic), in this individual's family member. This intronic variant is present in this individual and classified as a Variant of Uncertain Significance. Benign, Likely Benign, and silent and intronic variants with no evidence towards pathogenicity, are not included in this report but a full list is available upon request.
- 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 network of genetic providers, please contact Invitae at clientservices@invitae.com, or visit www.nsgc.org or tagc.med.sc.edu/professional_organizations.asp.

Complete Results

Gene	Variant	Zygosity	Variant Classification
ATM	c.2250G>A (Silent)	heterozygous	PATHOGENIC
BARD1	c.944C>T (p.Pro315Leu)	heterozygous	Uncertain Significance

The following genes were evaluated for sequence changes and exonic deletions/duplications:
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, DICER1, EPCAM (Deletion/duplication testing only), GREM1 (Promoter region deletion/duplication testing only), KIT, MEN1, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL

The following genes were evaluated for sequence changes only:
SDHA

Results are negative unless otherwise indicated

Benign, Likely Benign, and silent and intronic variants with no evidence towards pathogenicity, are not included in this report but are available upon request.

Variant Details

ATM, Exon 14, c.2250G>A (Silent), heterozygous, PATHOGENIC

- This sequence change affects codon 750 of the ATM mRNA. It is a 'silent' change, meaning that it does not change the encoded amino acid sequence of the ATM protein. It also falls at the last nucleotide of exon 14 of the ATM coding sequence.
- This variant is present in population databases (rs1137887, ExAC 0.002%).
- This variant has been reported in the literature in individuals and families affected with ataxia-telangiectasia (PMID: 9463314,10980530, 9887333, 10330348, 19691550). Exon 14 is also referred to as exon 16 in the literature. ClinVar contains an entry for this variant (Variation ID: 3044).
- Experimental studies evaluating the splicing effect of this variant reported that it resulted in skipping of exon 14 (c.2125_2250del, p.Ile709_Lys750del) from the ATM mRNA (PMID: 9887333, 10330348).
- For these reasons, this variant has been classified as Pathogenic.

BARD1, Exon 4, c.944C>T (p.Pro315Leu), heterozygous, Uncertain Significance

- This sequence change replaces proline with leucine at codon 315 of the BARD1 protein (p.Pro315Leu). The proline residue is moderately conserved and there is a moderate physicochemical difference between proline and leucine.
- This variant is present in population databases (rs148760338, ExAC 0.02%) but has not been reported in the literature in individuals with a BARD1-related disease. ClinVar contains an entry for this variant (Variation ID: 187028).
- 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 leucine 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.

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, Array CGH. Array CGH confirmation of NGS CNV calling performed by Invitae Corporation (475 Brannan Street, San Francisco, CA, 94107, #05D2040778). ARX trinucleotide repeat (Exon 2) confirmatory testing performed at Greenwood Genetic Center (101 Gregor Mendel Cir, Greenwood, SC 29646. #42D0689473). 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 Sanger sequencing of the relevant exon in both long-range amplicons. If a CNV variant is identified, MLPA 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 Sanger sequenced from the long-range amplicon to disambiguate the location of the CNV. Technical component of confirmatory sequencing is performed by Invitae Corporation (458 Brannan Street, San Francisco CA 94107. #05D2094793).
- A portion of the analytic workflow, sequencing of DNA libraries, was performed at the Invitae clinical core sequencing facility (CLIA #05D2094793) located at 458 Brannan St, San Francisco, CA, 94107.
- The following transcripts were used in this analysis: APC (NM_000038.5), ATM (NM_000051.3), AXIN2 (NM_004655.3), BARD1 (NM_000465.3), BMPR1A (NM_004329.2), BRCA1 (NM_007294.3), BRCA2 (NM_000059.3), BRIP1 (NM_032043.2), CDH1 (NM_004360.3), CDKN2A (NM_000077.4), CHEK2 (NM_007194.3), DICER1 (NM_177438.2), EPCAM (NM_002354.2: Deletion/duplication testing only), GREM1 (NM_013372.6: Promoter region deletion/duplication testing only), KIT (NM_000222.2), MEN1 (NM_130799.2), MLH1 (NM_000249.3), MSH2 (NM_000251.2), MSH6 (NM_000179.2), MUTYH (NM_001128425.1), NBN (NM_002485.4), NF1 (NM_000267.3), PALB2 (NM_024675.3), PDGFRA (NM_006206.4), PMS2 (NM_000535.5), POLD1 (NM_002691.3), POLE (NM_006231.3), PTEN (NM_000314.4), RAD50 (NM_005732.3), RAD51C (NM_058216.2), RAD51D (NM_002878.3), SDHA (NM_004168.3), SDHB (NM_003000.2), SDHC (NM_003001.3), SDHD (NM_003002.3), SMAD4 (NM_005359.5), SMARCA4 (NM_001128849.1), STK11 (NM_000455.4), TP53 (NM_000546.5), TSC1 (NM_000368.4), TSC2 (NM_000548.3), VHL (NM_000551.3).
- A PMID is a unique identifier referring to a published, scientific paper. Search by PMID at http://www.ncbi.nlm.nih.gov/pubmed.

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- 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) 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/.

Limitations

- This assay achieves >99% sensitivity and specificity for single nucleotide variants and insertions and deletions <15bp indels, based on validation study results. Sensitivity to detect insertions and deletions larger than 15bp but smaller than a full exon may be marginally reduced. Expansions and contractions within trinucleotide repeat regions may not be detected unless specified. Invitae's deletion/duplication analysis determines copy number with high confidence at >95% of targeted exons. Novel sequence changes in the promoter region and other non-coding regions will not be detected by this assay. This methodology may not detect low-level mosaicism. This report reflects the analysis of an extracted 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.
- CDKN2A: Analysis supports interpretation of the p16 protein only.

This report has been reviewed and approved by:

Placeholder for signature

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.

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