INVITAE

INVITAE PROACTIVE TESTING RESULTS

Patient name:	**Sample type:**	**Report date:**
DOB:	**Sample collection date:**	**Invitae #:**
Sex assigned at birth:	**Sample accession date:**	**Clinical team:**
Gender:		
Patient ID (MRN):		

Test performed

Sequence analysis and deletion/duplication testing of the 65 genes listed in the Genes Analyzed section.

- Invitae Cancer Screen

RESULT: CARRIER

A single clinically significant genetic change was found in the MUTYH gene, which is associated with being a carrier for a hereditary condition.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
MUTYH	c.536A>G (p.Tyr179Cys)	heterozygous	PATHOGENIC

About this test

This test evaluates 65 genes for variants (genetic changes) that indicate a significantly increased risk of developing certain types of cancer. These are disorders for which effective medical interventions and preventive measures are known and available. Genetic changes of uncertain significance are not included in this report; however, if additional evidence becomes available to indicate that a previously uncertain genetic change is clinically significant, Invitae will update this report and provide notification.

Next steps

- A carrier result is medically important and you should consider discussing this with an appropriate healthcare provider. While this result indicates you do not have any personal genetic risk factors for the conditions associated with the tested genes, it is important to note that at least a baseline, population-level risk remains for developing these types of disorders and age-appropriate screenings are still recommended. A complete family history may point to health risks not evaluated by this test. Genetic counseling is recommended to discuss the implications of this result and potential next steps.
- 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 Pathogenic variant, c.536A>G (p.Tyr179Cys), was identified in MUTYH.

- If a person carries two clinically significant genetic changes, one in each copy of the MUTYH gene, this may increase the risk for an autosomal recessive condition known as MUTYH-associated polyposis (MAP).
- This result is associated with being a carrier of autosomal recessive MAP. Carriers typically do not have symptoms of the condition, but may be at risk to have a child with the condition.
- MAP is a condition that significantly increases the risk to develop colon polyps and colon cancer. Screening and management guidelines exist to help prevent these cancers and/or identify them at an earlier stage. It is important to recognize that this result is not a diagnosis of cancer.

  Having a clinically significant genetic change in one copy of the MUTYH gene is common among individuals with Northern European ancestry. Studies suggest that individuals with one clinically significant genetic change in MUTYH may have a marginally increased risk of developing colon cancer when compared to individuals in the general population.
- Since genetic changes are often shared within families, there is a chance that biological relatives may be a carrier or at risk for autosomal recessive MAP and could consider testing. The chance of having a child with autosomal recessive MAP depends on whether this individual's partner is also a carrier of the same condition.

Variant details

MUTYH, Exon 7, c.536A>G (p.Tyr179Cys), heterozygous, PATHOGENIC

- This sequence change replaces tyrosine, which is neutral and polar, with cysteine, which is neutral and slightly polar, at codon 179 of the MUTYH protein (p.Tyr179Cys).
- This variant is present in population databases (rs34612342, gnomAD 0.2%), and has an allele count higher than expected for a pathogenic variant.
- This variant is a known common cause of MUTYH-associated polyposis (PMID: 23035301). This variant has been reported to co-segregate with disease in individuals affected with colorectal cancer and polyposis (with polyp numbers ranging from 10 to >100) (PMID: 12606733, 16557584, 17489848, 19793053, 21063410, 24444654).
- MUTYH-related conditions are inherited in an autosomal recessive fashion. However, there is evidence that monoallelic pathogenic MUTYH variants including this particular variant are associated with increased risk of colon cancer (PMID: 16492921, 19394335, 21171015, 24444654).
- This variant is also known as c.494A>G (p.Tyr165Cys).
- ClinVar contains an entry for this variant (Variation ID: 5293).
- An algorithm developed to predict the effect of missense changes on protein structure and function (PolyPhen-2) suggests that this variant is likely to be disruptive.
- Experimental studies have shown that this missense change affects MUTYH function (PMID: 11818965, 18534194, 19953527, 20848659).
- For these reasons, this variant has been classified as Pathogenic.

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Genes analyzed

This table represents a complete list of genes analyzed for this individual. Genes listed in this table may also have additional reported clinical associations outside of the conditions listed. Additional information about gene-condition associations can be found at http://www.omim.org. An asterisk (*) indicates that this gene has a limitation. Please see the Limitations section for details.

Cancer-related genes

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
AIP	NM_003977.3	Familial isolated pituitary adenoma (FIPA)
APC*	NM_000038.5	Colorectal, Endocrine, Gastric, Nervous System/ Brain and Pancreatic Cancer, Sarcoma
ATM*	NM_000051.3	Breast, Ovarian, Pancreatic, Prostate Cancer
AXIN2	NM_004655.3	Colorectal Cancer
BAP1	NM_004656.3	BAP1 tumor predisposition syndrome
BARD1	NM_000465.3	Breast Cancer
BMPR1A	NM_004329.2	Colorectal, Gastric and Pancreatic Cancer
BRCA1	NM_007294.3	Breast, Gynecologic, Pancreatic and Prostate Cancer
BRCA2	NM_000059.3	Breast, Gynecologic, Pancreatic and Prostate Cancer, Melanoma
BRIP1	NM_032043.2	Breast and Gynecologic Cancer
CDC73	NM_024529.4	Endocrine and Renal/Urinary Tract Cancer
CDH1	NM_004360.3	Breast, Colorectal and Gastric Cancer
CDK4	NM_000075.3	Melanoma
CDKN1B	NM_004064.4	Multiple endocrine neoplasia type 4 (MEN4)
CDKN2A (p14ARF)	NM_058195.3	Nervous System/Brain Cancer, Melanoma
CDKN2A (p16INK4a)	NM_000077.4	Pancreatic Cancer, Melanoma
CHEK2	NM_007194.3	Breast, Colorectal, Endocrine, Prostate Cancer
DICER1*	NM_177438.2	Endocrine, Gynecologic, Nervous System/Brain and Renal/Urinary Tract, Lung Cancer, Sarcoma
EGFR	NM_005228.3	EGFR-related predisposition to lung cancer, Neonatal inflammatory skin and bowel disease
EPCAM*	NM_002354.2	Colorectal, Gastric, Gynecologic, Nervous System/ Brain, Pancreatic, Prostate and Renal/Urinary Tract Cancer
FH*	NM_000143.3	Renal/Urinary Tract, Endocrine Cancer
FLCN	NM_144997.5	Renal/Urinary Tract Cancer
GREM1*	NM_013372.6	Colorectal Cancer
HOXB13	NM_006361.5	Prostate Cancer
KIT	NM_000222.2	Gastrointestinal Tumor or Cancer, Blood Cancer
LZTR1	NM_006767.3	Noonan spectrum disorders (NSDs) / RASopathies, Schwannomatosis
MAX*	NM_002382.4	Endocrine Cancer
MEN1*	NM_130799.2	Endocrine, Nervous System/Brain and Pancreatic Cancer
MET*	NM_001127500.1	Renal/Urinary Tract Cancer
MITF	NM_000248.3	Melanoma
MLH1*	NM_000249.3	Colorectal, Gastric, Gynecologic, Nervous System/ Brain, Pancreatic, Prostate and Renal/Urinary Tract Cancer
MSH2*	NM_000251.2	Colorectal, Gastric, Gynecologic, Nervous System/ Brain, Pancreatic, Prostate and Renal/Urinary Tract Cancer
MSH3*	NM_002439.4	Colorectal Cancer
MSH6*	NM_000179.2	Colorectal, Gastric, Gynecologic, Nervous System/ Brain, Pancreatic, Prostate and Renal/Urinary Tract Cancer
MUTYH	NM_001128425.1	Colorectal Cancer
NF1*	NM_000267.3	Breast, Endocrine, Gastric and Nervous System/ Brain Cancer
NF2	NM_000268.3	Nervous System/Brain Cancer
NTHL1	NM_002528.6	Colorectal Cancer
PALB2	NM_024675.3	Breast and Pancreatic Cancer
PDGFRA	NM_006206.4	Gastrointestinal Tumor or Cancer
PMS2*	NM_000535.5	Colorectal, Gastric, Gynecologic, Nervous System/ Brain, Pancreatic, Prostate and Renal/Urinary Tract Cancer
POLD1*	NM_002691.3	Colorectal Cancer
POLE	NM_006231.3	Colorectal Cancer
POT1	NM_015450.2	POT1 tumor predisposition syndrome
PRKAR1A	NM_002734.4	Endocrine and Nervous System/Brain Cancer
PTCH1	NM_000264.3	Nervous System/Brain and Skin Cancer
PTEN*	NM_000314.4	Breast, Colorectal, Endocrine, Gynecologic, Nervous System/Brain and Renal/Urinary Tract Cancer, Melanoma
RAD51C	NM_058216.2	Breast, and Gynecologic Cancer
RAD51D	NM_002878.3	Breast, and Gynecologic Cancer
RB1*	NM_000321.2	Melanoma, Retinoblastoma, Sarcoma
RET	NM_020975.4	Endocrine Cancer
SDHA*	NM_004168.3	Endocrine, Gastrointestinal Tumor or Cancer

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GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
SDHAF2	NM_017841.2	Endocrine Cancer
SDHB	NM_003000.2	Endocrine, Gastrointestinal Tumor or Cancer, Renal/ Urinary Tract Cancer
SDHC*	NM_003001.3	Endocrine, Gastrointestinal Tumor or Cancer, Renal/ Urinary Tract Cancer
SDHD	NM_003002.3	Endocrine, Gastrointestinal Tumor or Cancer, Renal/ Urinary Tract Cancer
SMAD4	NM_005359.5	Colorectal, Gastric and Pancreatic Cancer
SMARCA4	NM_001128849.1	Gynecologic Cancer
SMARCB1	NM_003073.3	Nervous System/Brain and Renal/Urinary Tract Cancer
STK11	NM_000455.4	Breast, Gastrointestinal, Gynecologic, Testicular, Lung, and Pancreatic Cancer
TMEM127	NM_017849.3	Endocrine Cancer
TP53	NM_000546.5	Breast, Endocrine, Gastrointestinal, Genitourinary, Gynecologic, Hematologic, Nervous System/Brain and Skin Cancer, Sarcoma
TSC1*	NM_000368.4	Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
TSC2	NM_000548.3	Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
VHL	NM_000551.3	Endocrine, Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
WT1	NM_024426.4	Renal/Urinary Tract Cancer

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 in the Genes Analyzed table. Enrichment and analysis focus on the coding sequence of the indicated transcripts, 20bp of flanking intronic sequence, 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. 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. Variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Confirmation of the presence and location of reportable variants is performed as needed based on stringent criteria using one of several validated orthogonal approaches (PubMed ID 30610921). Sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778). Confirmatory sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778). RNA sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778).

  The following additional 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. For C9orf72 repeat expansion testing, hexanucleotide repeat units are detected by repeat-primed PCR (RP-PCR) with fluorescently labeled primers followed by capillary electrophoresis. Interpretation Reference Ranges: Benign (Normal Range): <25 repeat units, Uncertain: 25-30 repeat units, Pathogenic (Full Mutation): >=31 repeat units (PMID: 21944779, 22406228, 23111906, 28689190, 31315673, 33168078, 33575483). A second round of RP-PCR utilizing a non-overlapping set of primers is used to confirm the initial call in the case of suspected allele sizes of 22 or more repeats. For RNA analysis of the genes indicated in the Genes Analyzed table, complementary DNA is synthesized by reverse transcription from RNA derived from a blood specimen and enriched for specific gene sequences using capture hybridization. After high-throughput sequencing using Illumina technology, the output reads are aligned to a reference sequence (genome build GRCh37; custom derivative of the RefSeq transcriptome) to identify the locations of exon junctions through the detection of split reads. The relative usage of exon junctions in a test specimen is assessed quantitatively and compared to the usage seen in control specimens. Abnormal exon junction usage is evaluated as evidence in the Sherloc variant interpretation framework. If an abnormal splicing pattern is predicted based on a DNA variant outside the typical reportable range, as described above, the presence of the variant is confirmed by targeted DNA sequencing.

- 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 in 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. While this test is intended to reflect the analysis of extracted genomic DNA from a referred patient, in very rare cases the analyzed DNA may not represent that individual's constitutional genome, such as in the case of a circulating hematolymphoid neoplasm, bone marrow transplant, blood transfusion, chimerism, culture artifact or maternal cell contamination. Interpretations are made on the assumption that any clinical information provided, including specimen identity, is accurate. Invitae's RNA analysis is not designed for use as a stand-alone diagnostic method and cannot determine absolute RNA levels. Results from the RNA analysis may not be informative for interpreting copy number gains.
APC: Sequencing analysis for exons 5 includes only cds +/- 10 bp. ATM: Sequencing analysis for exons 6, 24, 43 includes only cds +/- 10 bp. DICER1: Sequencing analysis for exons 22 includes only cds +/- 10 bp. EPCAM: Sequencing analysis is not offered for this gene. FH: Sequencing analysis for exons 9 includes only cds +/- 10 bp. MAX: Sequencing analysis for exons 2 includes only cds +/- 10 bp. MEN1: Sequencing analysis for exons 2 includes only cds +/- 10 bp. MET: Sequencing analysis for exons 12 includes only cds +/- 10 bp. MLH1: Sequencing analysis for exons 12 includes only cds +/- 10 bp. MSH2: Analysis includes the exon 1-7 inversion (Boland mutation). Sequencing analysis for exons 2, 5 includes only cds +/- 10 bp. MSH6: Sequencing analysis for exons 7, 10 includes only cds +/- 10 bp. PMS2: Sequencing analysis for exons 7 includes only cds +/- 10 bp. PTEN: Sequencing analysis for exons 8 includes only cds +/- 10 bp. RB1: Sequencing analysis for exons 15-16 includes only cds +/- 10 bp. SDHA: Deletion/duplication analysis is not offered for this gene and sequencing analysis is not offered for exon 14. Sequencing analysis for exons 6-8 includes only cds +/- 10 bp. SDHC: Sequencing analysis for exons 2, 6 includes only cds +/- 10 bp. TSC1: Sequencing analysis for exons 21 includes only cds +/- 10 bp. GREM1: Promoter region duplication testing only. POLD1: Sequencing analysis for exons 22 includes only cds +/- 10 bp. MSH3: Sequencing analysis of the repeat region of exon 1 (5:79950697-79950765) is not offered. NF1: Sequencing analysis for exons 2, 7, 25, 41, 48 includes only cds +/- 10 bp.

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:

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