INVITAE

# INVITAE DIAGNOSTIC TESTING RESULTS

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|---|---|---|---|---|---|
| **Patient name:** | Daria Lvova | **Sample type:** | Saliva | **Report date:** | 12/21/2020 |
| **DOB:** | 04/14/2011 | **Sample collection date:** | 12/04/2020 | **Invitae #:** | RQ1843997 |
| **Sex:** | Female | **Sample accession date:** | 12/09/2020 | **Clinical team:** | Tzviya Haziz<br>Shay Ben Shacher |
| **MRN:** | 755045911 | | | | |

**Reason for testing**

Diagnostic test for a personal history of disease

**Test performed**

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

## RESULT: POSITIVE

**One Pathogenic variant identified in RET. RET is associated with autosomal dominant multiple endocrine neoplasia and nonsyndromic Hirschsprung disease.**

| GENE | VARIANT | ZYGOSITY | VARIANT CLASSIFICATION |
|---|---|---|---|
| RET | c.2671T>G (p.Ser891Ala) | heterozygous | PATHOGENIC |

**About this test**

This diagnostic test evaluates 1 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.
- 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.2671T>G (p.Ser891Ala), was identified in RET.

- The RET gene is associated with autosomal dominant multiple endocrine neoplasia type 2 (MEN2) syndrome (MedGen UID: 9958) and non-syndromic Hirschsprung disease (MedGen UID: 419188).
- This result is consistent with a predisposition to, or diagnosis of, RET-related conditions.
- Pathogenic gain-of-function (GOF) RET variants cause MEN2, a condition associated with cancers of the endocrine glands that has three clinical subtypes: MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC). MEN2A is characterized by medullary thyroid carcinoma (MTC) (approximately 95%), pheochromocytoma (50% of cases) and hyperparathyroidism (approximately 20-30%). MEN2B is associated with MTC (nearly 100% of cases), pheochromocytoma (approximately 50%), while hyperparathyroidism is rare. The only clinical manifestation of FMTC is MTC (PMID: 23455356, 8918855, 19469690, 17895320). Some individuals with GOF RET variants located in exon 10 may have MEN2A and Hirschsprung disease, and therefore should have clinical evaluation for both conditions (PMID: 6136579, 10235148,15741265, 16356097, 25810047, 19958926, 25810047).
- Biological relatives have a chance of being at risk for RET-related conditions and should consider testing if clinically appropriate.

## Variant details

RET, Exon 15, c.2671T>G (p.Ser891Ala), heterozygous, PATHOGENIC

- This sequence change replaces serine with alanine at codon 891 of the RET protein (p.Ser891Ala). The serine residue is highly conserved and there is a moderate physicochemical difference between serine and alanine.
- This variant is not present in population databases (ExAC no frequency).
- This variant has been reported in numerous individuals and families affected with medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), and parathyroid hyperplasia (PMID: 20554711, 9398735, 24845513, 24449023, 23295303). According to the management guidelines of the American Thyroid Association (ATA), this variant is categorized as a moderate risk variant for MTC with ~10% incidence of PHEO or hyperparathyroidism (HPTH) (PMID: 25810047, 19469690, 11739416). ClinVar contains an entry for this variant (Variation ID: 13951).
- Experimental studies have shown that this missense change increases the phosphorylation levels of downstream effectors such as Akt and STAT3, and has a moderate transforming activity in vitro (PMID: 10445857, 17209045, 26356818).
- For these reasons, this variant has been classified as Pathogenic.

## 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. Results are negative 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 |
|---|---|
| RET | NM_020975.4 |

## 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. 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. 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). Technical component of Fibroblast cell-culturing and gDNA extraction from skin punch biopsy is performed by Invitae Corporation (5 Technology Drive, Irvine CA 92618, #05D1052995).
- 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/**.
- 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 (such as circulating hematolymphoid neoplasm, bone marrow transplant, recent blood transfusion, or maternal cell contamination), the analyzed DNA may not represent the patient's constitutional genome.

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## 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:

Matteo Vatta, Ph.D., FACMG
Clinical Molecular Geneticist

INVITAE

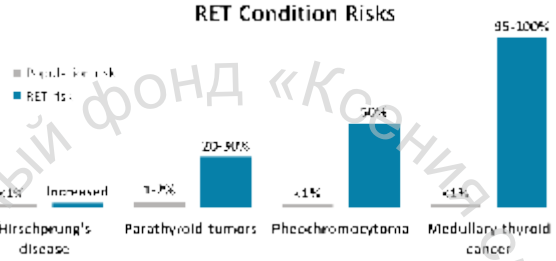
GENERAL GUIDELINES
# POSITIVE RESULTS GUIDE: RET

This document is not part of Invitae's clinical report and does not represent medical advice. These are general guidelines that are not specific to your result. You can use this guide to talk to your healthcare provider about your test results, clinical history, and the most current guidelines.

## What is a positive RET result?

A positive test result means that you have a genetic change, called a pathogenic or likely pathogenic variant ("mutation"), in your RET gene. Variants in RET can cause multiple endocrine neoplasia type 2 (MEN2) or Hirschsprung disease. Your result report may provide more information on which condition applies to your variant.

## What does this mean?




Some positive variants in the RET gene cause MEN2 and others cause Hirschsprung disease. Your result report may provide more information on which condition applies to your variant. It's possible for anyone to get cancer at some point in their life, however, people with MEN2 are more likely to get a specific type of thyroid cancer, called medullary thyroid cancer (MTC) than the average person. They may also develop noncancerous adrenal gland tumors (small glands found on top of each kidney) and overactive parathyroid glands (four small glands in the neck near the thyroid gland), usually caused by benign parathyroid tumors. Tumors on the adrenal glands (pheochromocytomas) can sometimes become cancerous. Some people with MEN2 may also have other physical differences such as being very tall and thin with hyperflexible joints. They may also have scoliosis (a curved spine) and neuromas (noncancerous tumors made of nerve cells) that can form in the intestines and on the lips and tongue. Certain variants in RET may cause a different condition called Hirschsprung disease, which is characterized by an absence of nerve cells from part of the large intestines (colon) causing constipation. Not everyone who is positive for RET will have all of these symptoms. See the table later in this guide for ways to find and manage MEN2 so you can seek treatment as soon as possible.

## What does this mean for family members?

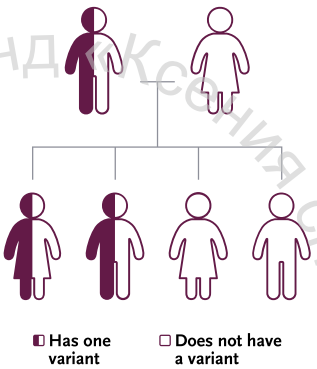
Genes and variants are passed from generation to generation. Your relatives may also have this variant in RET. Both men and women can inherit and pass on this variant.

**Who should be tested next?**
Your close relatives have a 50% chance of also having the same positive variant. This means your parents, siblings, and children. Your other relatives may also have this RET variant.

Inheriting this RET variant does not mean that a person will definitely develop RET-related symptoms. Variants in the RET gene affect everyone differently. Family members with the variant may develop symptoms at different ages, or they may develop different features.

Genetic testing is a personal choice and your family members may choose not to have genetic testing. It is recommended that they talk with their own healthcare provider about a plan for screening.

**Chance for passing on a variant**




## Create a plan with your healthcare provider

These options are a guide for you and your healthcare provider. They are meant to be used along with your genetic test results and other health information. Each option may or may not be right for you. Your positive test result on its own can not predict how this condition may affect you. Please talk with your healthcare provider to make a plan that's right for you.

**Options you and your healthcare provider might consider**

| CONDITION | RISK FOR GENERAL POPULATION | RISK FOR RET | OPTION | MORE INFORMATION |
|---|---|---|---|---|
| Medullary thyroid cancer | <1% | 95-100% | • Screening blood tests which may include calcitonin and carcinoembryonic antigen (CEA) levels (2) | • Helps find cancer so you can seek treatment as soon as possible.<br>• Results from this test and your personal and family health history will help determine if, when, and how often, to consider this option. |
| | | | • Neck ultrasound of thyroid and lymph nodes (2) | • Helps find cancer so you can seek treatment as soon as possible.<br>• Results from this test and your personal and family health history will help determine if, when, and how often, to consider this option. |
| | | | • Thyroidectomy (surgery to remove the thyroid) may be recommended between age 1-5, or at the time of diagnosis based on the genetic variant and personal health history (2) | • Help prevent thyroid cancer.<br>• Your personal and family health history will help determine if and when to consider this option. |
| Pheochromocytoma | <1% | 50% | • Screening blood and urine tests starting at age 11-16 or at the time of diagnosis may include plasma free or 24-hour urinary fractionated metanephrines (3) | • Helps find tumors so you can seek treatment as soon as possible.<br>• Results from this test and your personal and family health history will help determine if, when, and how often, to consider this option. |
| | | | • Chest CT scan with or without contrast and abdominal and pelvic CT scan or MRI (3) | • Helps find tumors so you can seek treatment as soon as possible.<br>• Results from this test and your personal and family health history will help determine if, when, and how often, to consider this option. |
| Parathyroid tumors | 1-2% | 20-30% | • Screening blood tests which may include serum calcium, parathyroid hormone, and 25-OH vitamin D (3) | • Helps find problems with the parathyroid so you can seek treatment as soon as possible.<br>• Results from these tests and your personal and family health history will help determine when, and how often, to consider this option. |
| | | | • Imaging tests such as neck ultrasound or CT scan (3) | • Helps find tumors so you can seek treatment as soon as possible.<br>• Results from this test and your personal and family health history will help determine if, when, and how often, to consider this option. |

These options outline recommendations from NCCN. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Thyroid Carcinoma V.2.2019 (2) and NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Neuroendocrine and Adrenal Tumors V1.2019 (3). © National Comprehensive Cancer Network, Inc. 2019. All rights reserved. Accessed December 27, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. We are always learning more about genetics and disease, so please always refer to the current guidelines and recommendations when considering surveillance and treatment options. Information in this document may not include all relevant international recommendations and acts as a supplement to the Invitae result report. This information is not meant to replace a discussion with your healthcare provider and should not be considered or interpreted as medical advice.

## We (and others) are here to help

Genetic counseling is recommended to help you clearly and accurately understand your results so it's important to talk to your genetic counselor or other healthcare provider about your test results. Invitae also has board-certified genetic counselors who are available to answer questions about your test results or these options. Log in to your patient portal (invitae.com) to view your results, search for a local or Invitae genetic counselor, or join Invitae's Patient Insight Network (PIN), a community where you can connect with other patients and share your experience.

## Notes for personalized assessment