INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

Patient name:	Vlad Luca Lupascu	**Sample type:**	Blood	**Report date:**	09/02/2021
DOB:	02/10/2002	**Sample collection date:**	07/27/2021	**Invitae #:**	RQ2611027
Sex:	Male	**Sample accession date:**	08/03/2021	**Clinical team:**	cristian minulescu
MRN:					

Reason for testing

Diagnostic test for a personal history of disease

Test performed

Sequence analysis and deletion/duplication testing of the 384 genes listed in the Genes Analyzed section.
Multiple panels/genes ordered: see Methods for complete list.

RE-REQUISITION REPORT: This report supersedes RQ2537352 (08.17.2021) and includes additional analyses.

RESULT: UNCERTAIN

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CPS1	c.1154T>C (p.Ile385Thr)	heterozygous	Uncertain Significance
ELAC2	c.1659G>A (Silent)	heterozygous	Uncertain Significance
HMGCS2	c.1220T>C (p.Ile407Thr)	heterozygous	Uncertain Significance
SLC7A13	c.1155A>T (p.Glu385Asp)	heterozygous	Uncertain Significance
SMPD1	c.106_107insCGCTGGCGCTGG (p.Leu35_Val36insAlaLeuAlaLeu)	heterozygous	Uncertain Significance
TMEM126B	c.133G>C (p.Asp45His)	heterozygous	Uncertain Significance
CHIT1	c.1049_1072dup (p.Trp358*)	heterozygous	Benign (reportable variant)
CHIT1	c.304G>A (p.Gly102Ser)	heterozygous	Benign (reportable variant)
GALC	c.1685T>C (p.Ile562Thr)	homozygous	Benign (Pseudodeficiency allele)

About this test

This diagnostic test evaluates 384 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.

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Next steps

- This test did not identify any pathogenic variants, but includes at least one result that is not completely understood at this time. Please note that the classification of variants may change over time as a result of new variant interpretation guidelines and/or new information. If an uncertain variant is reclassified, Invitae will update this report with the new interpretation and provide notification. This result 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. Clinical follow up may still be warranted. This result should be interpreted within the context of additional laboratory results, family history and clinical findings.

- One or more variants were identified that are not known to cause disease. See the CHIT1 and GALC variant(s) in the Variant Details section for more information.

- 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.

Clinical summary

A Variant of Uncertain Significance, c.1154T>C (p.Ile385Thr), was identified in CPS1.

- The CPS1 gene is associated with autosomal recessive carbamoyl phosphate synthetase I (CPS1) deficiency (MedGen UID: 199727).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.1659G>A (Silent), was identified in ELAC2.

- The ELAC2 gene is associated with autosomal recessive combined oxidative phosphorylation deficiency 17 (COXPD17) (MedGen UID: 815856, 1668540).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.1220T>C (p.Ile407Thr), was identified in HMGCS2.

- The HMGCS2 gene is associated with autosomal recessive 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase deficiency (MedGen UID: 414399).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.1155A>T (p.Glu385Asp), was identified in SLC7A13.

- The SLC7A13 gene currently has no well-established disease association; however, there is preliminary evidence supporting a correlation with an autosomal recessive neurodevelopmental condition (PMID: 22494076).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.106_107insCGCTGGCGCTGG (p.Leu35_Val36insAlaLeuAlaLeu), was identified in SMPD1.

- The SMPD1 gene is associated with autosomal recessive acid sphingomyelinase (ASM) deficiency, which includes Niemann-Pick disease type A (MedGen UID: 78650) and Niemann-Pick disease type B (MedGen UID: 78651).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.133G>C (p.Asp45His), was identified in TMEM126B.

- The TMEM126B gene is associated with autosomal recessive mitochondrial complex I deficiency (MedGen UID: 1648451).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/family.

Variant details

CPS1, Exon 11, c.1154T>C (p.Ile385Thr), heterozygous, Uncertain Significance

- This sequence change replaces isoleucine with threonine at codon 385 of the CPS1 protein (p.Ile385Thr). The isoleucine residue is weakly conserved and there is a moderate physicochemical difference between isoleucine and threonine.
- This variant is present in population databases (rs201955205, ExAC 0.03%).
- This variant has not been reported in the literature in individuals with CPS1-related disease.
- 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 threonine 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 and their clinical significance is uncertain.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

ELAC2, Exon 17, c.1659G>A (Silent), heterozygous, Uncertain Significance

- This sequence change affects codon 553 of the ELAC2 mRNA. It is a 'silent' change, meaning that it does not change the encoded amino acid sequence of the ELAC2 protein.
- This variant is present in population databases (rs200420215, ExAC 0.1%).
- This variant has not been reported in the literature in individuals with ELAC2-related conditions. ClinVar contains an entry for this variant (Variation ID: 241273).
- Algorithms developed to predict the effect of sequence changes on RNA splicing suggest that this variant may disrupt the consensus splice site, but this prediction has not been confirmed by published transcriptional studies.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

HMGCS2, Exon 7, c.1220T>C (p.Ile407Thr), heterozygous, Uncertain Significance

- This sequence change replaces isoleucine with threonine at codon 407 of the HMGCS2 protein (p.Ile407Thr). The isoleucine residue is highly conserved and there is a moderate physicochemical difference between isoleucine and threonine.
- This variant is present in population databases (rs766898190, ExAC 0.005%).
- This missense change has been observed in individual(s) with clinical features of HMG-CoA synthase deficiency (PMID: 25511235).
- Algorithms developed to predict the effect of missense changes on protein structure and function output the following: SIFT: "Deleterious"; PolyPhen-2: "Benign"; Align-GVGD: "Class C25". The threonine amino acid residue is found in multiple mammalian species, which suggests that this missense change does not adversely affect protein function.
- Experimental studies have shown that this missense change affects HMGCS2 function (PMID: 29597274).
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

SLC7A13, Exon 3, c.1155A>T (p.Glu385Asp), heterozygous, Uncertain Significance

- This sequence change replaces glutamic acid with aspartic acid at codon 385 of the SLC7A13 protein (p.Glu385Asp). The glutamic acid residue is moderately conserved and there is a small physicochemical difference between glutamic acid and aspartic acid.
- This variant is present in population databases (rs202015119, ExAC 0.008%).
- This variant has not been reported in the literature in individuals affected with SLC7A13-related conditions.
- Algorithms developed to predict the effect of missense changes on protein structure and function (SIFT, PolyPhen-2, Align-GVGD) all suggest that this variant is likely to be tolerated.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

SMPD1, Exon 1, c.106_107insCGCTGGCGCTGG (p.Leu35_Val36insAlaLeuAlaLeu), heterozygous, Uncertain Significance

- This variant, c.106_107insCGCTGGCGCTGG, results in the insertion of 4 amino acid(s) to the SMPD1 protein (p.Leu35_Val36insAlaLeuAlaLeu), but otherwise preserves the integrity of the reading frame.
- This variant is not present in population databases (ExAC no frequency).
- This variant has not been reported in the literature in individuals affected with SMPD1-related conditions.
- Experimental studies and prediction algorithms are not available or were not evaluated, and the functional significance of this variant is currently unknown.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

TMEM126B, Exon 2, c.133G>C (p.Asp45His), heterozygous, Uncertain Significance

- This sequence change replaces aspartic acid with histidine at codon 45 of the TMEM126B protein (p.Asp45His). The aspartic acid residue is moderately conserved and there is a moderate physicochemical difference between aspartic acid and histidine.
- This variant is not present in population databases (ExAC no frequency).
- This variant has not been reported in the literature in individuals affected with TMEM126B-related conditions.
- Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Deleterious"; PolyPhen-2: "Probably Damaging"; Align-GVGD: "Class C0").
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

CHIT1, Exon 10, c.1049_1072dup (p.Trp358*), heterozygous, Benign (reportable variant)

- Chitotriosidase enzymatic activity is a prognostic and therapeutic biomarker for certain lysosomal storage disorders, such as Gaucher and Niemann-Pick disease A/B/C (PMID: 8750610, 15669690, 17869233).
- This variant is present at very high frequency in population databases (57% in the East Asian population in gnomAD)
- An increasing body of evidence exists for its utility as biomarker for other, non-lysosomal, disorders associated with inflammation and macrophage activation, including sarcoidosis (PMID: 24594143, 31906975), interstitial lung disease (PMID: 31092718, 17631992), and neuroinflammatory or neurodegenerative disorders (PMID: 22014002, 25563799).
- ClinVar contains an entry for this variant (Variation ID: 294920).
- The c.1049_1072dup variant has been shown to lead to aberrant mRNA and result in an inactive human chitotriosidase enzyme (PMID: 7592832, 9748235).
- While chitotriosidase deficiency is not associated with any human disease, the presence of this variant makes chitotriosidase enzymatic activity an unreliable biomarker. For these reasons, this variant is classified as a Benign Reportable Variant.

CHIT1, Exon 4, c.304G>A (p.Gly102Ser), heterozygous, Benign (reportable variant)

- Chitotriosidase enzymatic activity is a prognostic and therapeutic biomarker for certain lysosomal storage disorders, such as Gaucher and Niemann-Pick disease A/B/C (PMID: 8750610, 15669690, 17869233).
- This variant is present at very high frequency in population databases (37% in gnomAD)
- An increasing body of evidence exists for its utility as biomarker for other, non-lysosomal, disorders associated with inflammation and macrophage activation, including sarcoidosis (PMID: 24594143, 31906975), interstitial lung disease (PMID: 31092718, 17631992), and neuroinflammatory or neurodegenerative disorders (PMID: 22014002, 25563799).
- ClinVar contains an entry for this variant (Variation ID: 9526).
- This missense variant has been shown to cause a 30-50% reduction in CHIT1 enzymatic activity both in vivo and in vitro (PMID: 19725875, 24060732).
- While chitotriosidase deficiency is not associated with any human disease, the presence of this variant makes chitotriosidase enzymatic activity an unreliable biomarker. For these reasons, this variant is classified as a Benign Reportable Variant.

GALC, Exon 15, c.1685T>C (p.Ile562Thr), homozygous, Benign (Pseudodeficiency allele)

- This sequence change replaces isoleucine with threonine at codon 562 of the GALC protein (p.Ile562Thr). The isoleucine residue is weakly conserved and there is a moderate physicochemical difference between isoleucine and threonine.
- This variant is present in population databases (rs398607, ExAC 61%).
- This variant is a known pseudodeficiency allele and individuals with this variant can exhibit low galactocerebrosidase activity during enzyme analysis. On its own, this variant mildly reduces enzyme activity. However, it has been shown to further reduce GALC enzyme activity when it is located on the same chromosome (in cis) with pathogenic GALC variants (PMID: 26795590, 26865610, 27126738, 27638593). Individuals with pseudodeficiency alleles may exhibit false positive results on related biochemical tests, but pseudodeficiency alleles are not known to cause disease. Although pseudodeficiency alleles do not cause disease, other carrier relatives may have abnormal enzyme testing.
- This variant is also known as p.Ile546Thr or p.I546T.
- ClinVar contains an entry for this variant (Variation ID: 92497).
- Advanced modeling of protein sequence and biophysical properties (such as structural, functional, and spatial information, amino acid conservation, physicochemical variation, residue mobility, and thermodynamic stability) performed at Invitae indicates that this missense variant is not expected to disrupt GALC protein function.
- For these reasons, this variant has been classified as a Benign pseudodeficiency allele.

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
AARS2	NM_020745.3
AASS	NM_005763.3
ABAT	NM_020686.5
ABCB7	NM_004299.4
ACACA	NM_198839.2
ACAD9	NM_014049.4
ACADM	NM_000016.5
ACADS	NM_000017.3
ACADVL	NM_000018.3
ACAT1	NM_000019.3
ACO2	NM_001098.2
ADAR	NM_001111.4
AFG3L2	NM_006796.2
AGA	NM_000027.3
AGK	NM_018238.3
AIFM1	NM_004208.3
AK2	NM_001625.3
ALAS2	NM_000032.4
ALDH3A2	NM_000382.2
AMPD1	NM_000036.2
AMT	NM_000481.3
APOPT1	NM_032374.4
APTX	NM_175073.2
ARSA	NM_000487.5
ARSB	NM_000046.3
ASAH1	NM_177924.3
ATP13A2	NM_022089.3
ATP5A1	NM_001001937.1
ATP5D	NM_001001975.1
ATP5E	NM_006886.3
ATP7B	NM_000053.3
ATPAF2	NM_145691.3
AUH	NM_001698.2
BAG3	NM_004281.3
BCS1L	NM_004328.4
BOLA3	NM_212552.2
BTD	NM_000060.3
C12orf65	NM_152269.4
C19orf12	NM_001031726.3
C19orf70	NM_205767.2
C1QBP	NM_001212.3
CA5A	NM_001739.1
CARS2	NM_024537.3
CEP89	NM_032816.4
CHAT	NM_020549.4
CHCHD10	NM_213720.2
CHIT1	NM_003465.2
CLN3	NM_001042432.1
CLN5	NM_006493.2
CLN6	NM_017882.2
CLN8	NM_018941.3
CLPB	NM_030813.5
CLPP	NM_006012.2
COA3	NM_001040431.2
COA5	NM_001008215.2
COA6	NM_001012985.2
COA7	NM_023077.2
COASY	NM_025233.6
COQ2	NM_015697.7
COQ4	NM_016035.4
COQ6	NM_182476.2
COQ7	NM_016138.4
COQ8A	NM_020247.4
COQ8B	NM_024876.3
COQ9	NM_020312.3
COX10*	NM_001303.3
COX14	NM_032901.3
COX15	NM_004376.6
COX20	NM_198076.5
COX4I2	NM_032609.2
COX6A1	NM_004373.3
COX6B1	NM_001863.4
COX7B	NM_001866.2
COX8A	NM_004074.2
CPS1	NM_001875.4
CPT1A	NM_001876.3
CPT2	NM_000098.2
CTNS	NM_004937.2
CTSA	NM_000308.3
CTSD	NM_001909.4
CTSF	NM_003793.3
CTSK	NM_000396.3
CYC1	NM_001916.4
CYCS	NM_018947.5
D2HGDH	NM_152783.4
DARS2	NM_018122.4
DES	NM_001927.3
DGUOK	NM_080916.2
DLAT	NM_001931.4
DLD	NM_000108.4
DNA2	NM_001080449.2
DNAJC19	NM_145261.3
DNAJC5	NM_025219.2
DNM1L	NM_012062.4
EARS2	NM_001083614.1
ECHS1	NM_004092.3
ELAC2	NM_018127.6
ETFA	NM_000126.3
ETFB	NM_001985.2
ETFDH	NM_004453.3
ETHE1	NM_014297.3
FARS2	NM_006567.3
FASTKD2	NM_014929.3
FBXL4	NM_012160.4
FDX2	NM_001031734.3

GENE	TRANSCRIPT
FH*	NM_000143.3
FLAD1	NM_025207.4
FOXRED1	NM_017547.3
FUCA1	NM_000147.4
GAA	NM_000152.3
GALC*	NM_000153.3
GALNS	NM_000512.4
GAMT	NM_000156.5
GARS	NM_002047.2
GATM	NM_001482.2
GCDH	NM_000159.3
GDAP1	NM_018972.2
GFER	NM_005262.2
GFM1	NM_024996.5
GFM2	NM_032380.4
GLA	NM_000169.2
GLB1	NM_000404.2
GLDC	NM_000170.2
GLRX5	NM_016417.2
GM2A	NM_000405.4
GNPTAB	NM_024312.4
GNPTG	NM_032520.4
GNS	NM_002076.3
GRN	NM_002087.3
GTPBP3	NM_133644.3
GUSB	NM_000181.3
GYG2	NM_003918.2
HADH	NM_005327.4
HADHA	NM_000182.4
HADHB	NM_000183.2
HARS2	NM_012208.3
HCCS	NM_005333.4
HEXA	NM_000520.4
HEXB	NM_000521.3
HGSNAT	NM_152419.2
HIBCH	NM_014362.3
HLCS	NM_000411.6
HMGCL	NM_000191.2
HMGCS2	NM_005518.3

GENE	TRANSCRIPT
HSD17B10	NM_004493.2
HSPD1	NM_002156.4
HTRA2	NM_013247.4
HYAL1	NM_153281.1
IARS2	NM_018060.3
IBA57	NM_001010867.3
IDH2	NM_002168.3
IDH3B	NM_006899.4
IDS*	NM_000202.6
IDUA	NM_000203.4
IFIH1	NM_022168.3
ISCA1	NM_030940.3
ISCA2	NM_194279.3
ISCU	NM_213595.3
KARS	NM_001130089.1
KCTD7	NM_153033.4
L2HGDH	NM_024884.2
LAMP2	NM_002294.2
LARS	NM_020117.10
LARS2	NM_015340.3
LIAS	NM_006859.3
LIPA	NM_000235.3
LIPT1	NM_145199.2
LIPT2	NM_001144869.2
LMBRD1	NM_018368.3
LONP1	NM_004793.3
LRPPRC	NM_133259.3
LYRM4	NM_020408.5
LYRM7	NM_181705.3
MAN2B1	NM_000528.3
MANBA	NM_005908.3
MARS2	NM_138395.3
MCOLN1	NM_020533.2
MECR	NM_016011.3
MFF*	NM_020194.5
MFN2	NM_014874.3
MFSD8	NM_152778.2
MGME1	NM_052865.3
MICU1	NM_006077.3

GENE	TRANSCRIPT
MIPEP	NM_005932.3
MPC1	NM_016098.3
MPV17	NM_002437.4
MRPL12	NM_002949.3
MRPL3	NM_007208.3
MRPL40	NM_003776.3
MRPL44	NM_022915.3
MRPS14	NM_022100.2
MRPS16	NM_016065.3
MRPS2	NM_016034.4
MRPS22	NM_020191.2
MRPS23	NM_016070.3
MRPS34	NM_001300900.1
MRPS7	NM_015971.3
MSTO1*	NM_018116.3
MTFMT	NM_139242.3
MTHFD1	NM_005956.3
MTO1	NM_012123.3
MTPAP	NM_018109.3
NADK2	NM_001085411.2
NAGA	NM_000262.2
NAGLU	NM_000263.3
NARS2	NM_024678.5
NAXE	NM_144772.2
NDUFA1	NM_004541.3
NDUFA10	NM_004544.3
NDUFA11	NM_175614.4
NDUFA12	NM_018838.4
NDUFA13	NM_015965.6
NDUFA2	NM_002488.4
NDUFA4	NM_002489.3
NDUFA6	NM_002490.4
NDUFA9	NM_005002.4
NDUFAF1	NM_016013.3
NDUFAF2	NM_174889.4
NDUFAF3	NM_199069.1
NDUFAF4	NM_014165.3
NDUFAF5	NM_024120.4
NDUFAF6	NM_152416.3

GENE	TRANSCRIPT
NDUFAF7	NM_001083946.1
NDUFB11*	NM_019056.6
NDUFB3	NM_002491.2
NDUFB8	NM_005004.3
NDUFB9	NM_005005.2
NDUFS1	NM_005006.6
NDUFS2	NM_004550.4
NDUFS3	NM_004551.2
NDUFS4	NM_002495.3
NDUFS6	NM_004553.4
NDUFS7	NM_024407.4
NDUFS8	NM_002496.3
NDUFV1	NM_007103.3
NDUFV2	NM_021074.4
NEU1	NM_000434.3
NFS1	NM_021100.4
NFU1	NM_001002755.2
NGLY1	NM_018297.3
NNT	NM_012343.3
NPC1	NM_000271.4
NPC2	NM_006432.3
NR2F1	NM_005654.5
NSUN3	NM_022072.3
NUBPL	NM_025152.2
NUP62	NM_153719.3
OGDH	NM_002541.3
OPA1	NM_015560.2;NM_130837.2
OPA3	NM_025136.3
OTC	NM_000531.5
OXCT1	NM_000436.3
PANK2	NM_153638.2
PARS2	NM_152268.3
PC	NM_000920.3
PCCA	NM_000282.3
PCCB	NM_000532.4
PCK2	NM_004563.3
PDHA1	NM_000284.3
PDHB	NM_000925.3
PDHX	NM_003477.2

GENE	TRANSCRIPT
PDK3	NM_001142386.2
PDP1	NM_018444.3
PDSS1	NM_014317.4
PDSS2	NM_020381.3
PET100	NM_001171155.1
PINK1	NM_032409.2
PITRM1	NM_001242309.1
PMPCA	NM_015160.2
PMPCB	NM_004279.2
PNKD	NM_015488.4
PNPLA8	NM_015723.4
PNPT1	NM_033109.4
POLG	NM_002693.2
POLG2	NM_007215.3
POP1	NM_015029.2
PPA2	NM_176869.2
PPOX	NM_000309.3
PPT1	NM_000310.3
PSAP	NM_002778.3
PUS1	NM_025215.5
QARS	NM_005051.2
QRSL1	NM_018292.4
RANBP2*	NM_006267.4
RARS*	NM_002887.3
RARS2	NM_020320.3
REEP1	NM_022912.2
RMND1	NM_017909.3
RNASEH1	NM_002936.4
RNASEH2A	NM_006397.2
RNASEH2B	NM_024570.3
RNASEH2C	NM_032193.3
RRM2B	NM_015713.4
SACS	NM_014363.5
SAMHD1	NM_015474.3
SARS2	NM_017827.3
SCN1A	NM_001165963.1
SCO1	NM_004589.3
SCO2	NM_005138.2
SDHA*	NM_004168.3

GENE	TRANSCRIPT
SDHAF1	NM_001042631.2
SDHB	NM_003000.2
SDHC*	NM_003001.3
SDHD	NM_003002.3
SERAC1	NM_032861.3
SFXN4	NM_213649.1
SGSH	NM_000199.3
SIRT1	NM_012238.4
SLC17A5	NM_012434.4
SLC19A2	NM_006996.2
SLC19A3	NM_025243.3
SLC22A5	NM_003060.3
SLC25A1	NM_005984.4
SLC25A12	NM_003705.4
SLC25A13	NM_014251.2
SLC25A15	NM_014252.3
SLC25A19	NM_021734.4
SLC25A20	NM_000387.5
SLC25A21	NM_030631.3
SLC25A22	NM_024698.5
SLC25A26*	NM_001164796.1
SLC25A3	NM_005888.3
SLC25A32	NM_030780.4
SLC25A38	NM_017875.2
SLC25A4	NM_001151.3
SLC25A42	NM_178526.4
SLC25A46	NM_138773.2
SLC39A8	NM_022154.5
SLC52A2	NM_024531.4
SLC52A3	NM_033409.3
SLC6A8	NM_005629.3
SLC7A13	NM_138817.2
SMPD1	NM_000543.4
SPAST	NM_014946.3
SPG7	NM_003119.3
STAT2	NM_005419.3
STXBP1	NM_003165.3
SUCLA2	NM_003850.2
SUCLG1	NM_003849.3

GENE	TRANSCRIPT
SUCLG2	NM_001177599.1
SUGCT	NM_024728.2
SUMF1	NM_182760.3
SURF1	NM_003172.3
TACO1	NM_016360.3
TANGO2	NM_152906.6
TARS2	NM_025150.4
TAZ	NM_000116.4
TFAM	NM_003201.2
TIMM50	NM_001001563.3
TIMM8A	NM_004085.3
TIMMDC1	NM_016589.3
TK2	NM_004614.4
TMEM126A	NM_032273.3
TMEM126B	NM_018480.4
TMEM70	NM_017866.5
TOP1MT	NM_052963.2
TOP3A	NM_004618.4
TPK1	NM_022445.3
TPP1	NM_000391.3
TRAP1	NM_016292.2
TREX1	NM_033629.4
TRIT1	NM_017646.5
TRMT10C	NM_017819.3
TRMT5	NM_020810.3
TRMU	NM_018006.4
TRNT1	NM_182916.2
TSFM*	NM_001172696.1
TTC19	NM_017775.3
TUFM	NM_003321.4
TWNK	NM_021830.4
TXN2	NM_012473.3
TYMP	NM_001953.4
UQCC2	NM_032340.3
UQCC3	NM_001085372.2
UQCRB	NM_006294.4
UQCRC2	NM_003366.3
UQCRQ	NM_014402.4
VARS2	NM_001167734.1

GENE	TRANSCRIPT
WARS2	NM_015836.3
WDR45	NM_007075.3
WFS1	NM_006005.3
XPNPEP3	NM_022098.3
YARS2	NM_001040436.2
YME1L1	NM_139312.2

Methods

- Complete list of tests performed: Invitae Comprehensive Lysosomal Storage Disorders Panel, Add-on Chitotriosidase Deficiency Gene, Add-on Preliminary Evidence Gene, Add-on Adult-onset Neuronal Ceroid Lipofuscinoses Genes, Invitae Nuclear Mitochondrial Disorders Panel
- 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, 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 (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. Confirmation of the presence and location of reportable variants is performed based on stringent criteria established by Invitae (1400 16th Street, San Francisco, CA 94103, #05D2040778), as needed, using one of several validated orthogonal approaches (PubMed ID 30610921). 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). 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. 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. RNA sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2094793). 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. 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. Invitae's RNA analysis is not designed for use as a stand-alone diagnostic method and cannot determine absolute RNA levels.

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. MSTO1: Deletion/duplication analysis is not offered for exons 1-7, 10, 12-14 and sequencing analysis is not offered for exons 1-7, 10, 13-14. RANBP2: Deletion/duplication and sequencing analysis is not offered for exons 1-11, 15-29. TSFM: Sequencing analysis is not offered for exon 5. MFF: Deletion/duplication analysis is not offered for exon 3. FH: Sequencing analysis for exons 9 includes only cds +/- 10 bp. IDS: Detection of complex rearrangements not offered (PMID: 7633410, 20301451). SLC25A26: Deletion/duplication analysis is not offered for exon 5. GALC: Deletion/duplication analysis is not offered for exon 6. COX10: Deletion/duplication and sequencing analysis is not offered for exon 6. RARS: Deletion/duplication analysis is not offered for exon 14. NDUFB11: Deletion/duplication and sequencing analysis is not offered for exon 1.

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:

Christina Y. Hung, MD, FACMG
Clinical Molecular and Biochemical Geneticist

INVITAE

VUS RESULTS GUIDE

What your results mean for you

No significant genetic changes ("pathogenic variants" or "mutations") were found in your genetic test. However, your test did find a genetic change called a variant of uncertain significance (VUS) in one or more of the genes tested. When we see a genetic change, but are unsure of its impact on health, it is called a variant of uncertain significance.

Right now, there is not enough information about the VUS to know whether it causes disease or not. A VUS is a common type of result. We all have many genetic changes that do not cause medical problems. Most of the time, we later learn that a VUS is not related to disease risk.

Your risk for disease could still be influenced by a combination of unidentified genetic, personal, lifestyle and/or environmental factors. So, it's important to talk to your healthcare provider if you have questions about your risk.

Create a plan with your healthcare provider

These genetic test results should be shared with your healthcare providers. The chance for you to develop a disease is not determined by genetic test results alone. Your provider can help you make informed decisions about your healthcare.

What your results mean for your family

Testing family members for a VUS is usually not recommended. However, your report will note if testing your family members will help us learn more about your specific VUS.

Although your genetic test did not find a significant genetic change, your family members have their own unique genetic makeup. Genetic testing can help them understand their overall chance of developing a genetic disease.

We (and others) are here to help

Genetic counseling can 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 your personal or family medical history.

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.

This information in this results guide is meant to be used along with your genetic test results and other health information. It is not meant to replace a discussion with your healthcare provider and should not be considered or interpreted as medical advice.

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