INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

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

Reason for testing

Diagnostic test for a personal history of disease

Test performed

Sequence analysis and deletion/duplication testing of the 429 genes listed in the Genes Analyzed section.

- Invitae Primary Immunodeficiency Panel

RESULT: UNCERTAIN

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CASP10	c.664A>G (p.Thr222Ala)	heterozygous	Uncertain Significance
IKBKB	c.1849G>A (p.Val617Ile)	heterozygous	Uncertain Significance
PSTPIP1	c.1183G>A (p.Gly395Ser)	heterozygous	Uncertain Significance
TFRC	c.821T>C (p.Leu274Ser)	heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 429 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.

Clinical comments

- When a single Variant of Uncertain Significance is found in a requisitioned gene that is only associated with autosomal recessive condition(s), it may not be included in the report.

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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.

- Testing of up to two family members for the Variant(s) of Uncertain Significance (VUS) identified in IKBKB is available at no additional cost. Please consider this individual's clinical features and availability of informative family members to test before ordering VUS resolution testing. More details on our VUS Resolution Program, including required documentation, 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.

Clinical summary

A Variant of Uncertain Significance, c.664A>G (p.Thr222Ala), was identified in CASP10.

- The CASP10 gene currently has no well-established disease association; however, there is preliminary evidence supporting a correlation with autosomal dominant and autosomal recessive autoimmune lymphoproliferative syndrome (ALPS-CASP10) (PMID: 10412980, 16446975).
- 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.1849G>A (p.Val617Ile), was identified in IKBKB.

- The IKBKB gene is associated with autosomal dominant anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) due to IKBKB gain-of-function (MedGen UID: 1648385) and autosomal recessive combined immunodeficiency (MedGen UID: 1648569).
- 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.
- This variant qualifies for complimentary family studies as part of our VUS Resolution Program. Familial VUS testing is recommended if informative family members are available and are likely to provide additional evidence for future variant reclassification. Details on our VUS Resolution Program can be found at https://www.invitae.com/family.

A Variant of Uncertain Significance, c.1183G>A (p.Gly395Ser), was identified in PSTPIP1.

- The PSTPIP1 gene is associated with autosomal dominant pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome (MedGen UID: 346801).
- 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.821T>C (p.Leu274Ser), was identified in TFRC.

- The TFRC gene currently has no well-established disease association; however, there is preliminary evidence supporting a correlation with autosomal recessive combined immunodeficiency due to TFRC deficiency (PMID: 26642240).
- 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

CASP10, Exon 5, c.664A>G (p.Thr222Ala), heterozygous, Uncertain Significance

- This sequence change replaces threonine, which is neutral and polar, with alanine, which is neutral and non-polar, at codon 222 of the CASP10 protein (p.Thr222Ala).
- This variant is present in population databases (rs763551197, gnomAD 0.03%).
- This variant has not been reported in the literature in individuals affected with CASP10-related conditions.
- ClinVar contains an entry for this variant (Variation ID: 661386).

- 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.

IKBKB, Exon 19, c.1849G>A (p.Val617Ile), heterozygous, Uncertain Significance

- This sequence change replaces valine, which is neutral and non-polar, with isoleucine, which is neutral and non-polar, at codon 617 of the IKBKB protein (p.Val617Ile).
- This variant is not present in population databases (gnomAD no frequency).
- This variant has not been reported in the literature in individuals affected with IKBKB-related conditions.
- 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 IKBKB protein function.
- 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.

PSTPIP1, Exon 15, c.1183G>A (p.Gly395Ser), heterozygous, Uncertain Significance

- This sequence change replaces glycine, which is neutral and non-polar, with serine, which is neutral and polar, at codon 395 of the PSTPIP1 protein (p.Gly395Ser).
- The frequency data for this variant in the population databases is considered unreliable, as metrics indicate poor data quality at this position in the gnomAD database.
- This variant has not been reported in the literature in individuals affected with PSTPIP1-related conditions.
- ClinVar contains an entry for this variant (Variation ID: 854353).
- 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 expected to disrupt PSTPIP1 protein function.
- 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.

TFRC, Exon 8, c.821T>C (p.Leu274Ser), heterozygous, Uncertain Significance

- This sequence change replaces leucine, which is neutral and non-polar, with serine, which is neutral and polar, at codon 274 of the TFRC protein (p.Leu274Ser).
- This variant is present in population databases (rs372946953, gnomAD 0.006%).
- This variant has not been reported in the literature in individuals affected with TFRC-related conditions.
- ClinVar contains an entry for this variant (Variation ID: 1400474).
- 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 TFRC protein function.
- 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.

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. An asterisk (*) indicates that this gene has a limitation. Please see the Limitations section for details. Results are negative unless otherwise indicated in the report. Benign and Likely Benign variants are not included in this report and in specific scenarios variants of uncertain significance in the requisitioned gene(s) may not be included in this report. These variants are available upon request.

GENE	TRANSCRIPT
ACD	NM_001082486.1
ACP5	NM_001111035.2
ACTB	NM_001101.3
ADA	NM_000022.2
ADA2	NM_001282225.1
ADAM17	NM_003183.5
ADAR	NM_001111.4
ADGRE2*	NM_013447.3
AICDA	NM_020661.2
AIRE	NM_000383.3
AK2*	NM_001625.3
ALG6	NM_013339.3
ALPK1*	NM_001102406.1
ANGPT1	NM_001146.4
ANKZF1	NM_018089.2
AP3B1	NM_003664.4
AP3D1	NM_001261826.1
ARHGEF1	NM_199002.1
ARPC1B	NM_005720.3
ASAH1	NM_177924.3
ATM*	NM_000051.3
ATP6AP1	NM_001183.5
B2M	NM_004048.2
BACH2	NM_021813.3
BCL10	NM_003921.4
BCL11B	NM_138576.3
BLM	NM_000057.3
BLNK	NM_013314.3
BLOC1S3	NM_212550.4
BLOC1S6	NM_012388.3
BTK	NM_000061.2
C17orf62	NM_001033046.3
C1QA	NM_015991.2
C1QB	NM_000491.3
C1QC	NM_172369.3
C1S	NM_201442.2
C2	NM_000063.5
C3	NM_000064.3
C5	NM_001735.2
C6	NM_000065.3
C7	NM_000587.2
C8A	NM_000562.2
C8B	NM_000066.3
C9	NM_001737.4
CARD11	NM_032415.5
CARD14	NM_024110.4
CARD8	NM_014959.3
CARD9	NM_052813.4
CARMIL2	NM_001013838.1
CASP10	NM_032977.3
CASP8	NM_001228.4
CBL	NM_005188.3
CCBE1	NM_133459.3
CD19	NM_001770.5
CD247	NM_198053.2
CD27	NM_001242.4
CD3D	NM_000732.4
CD3E	NM_000733.3
CD3G	NM_000073.2
CD40	NM_001250.5
CD40LG	NM_000074.2
CD46	NM_002389.4
CD55	NM_000574.4
CD59	NM_203330.2
CD79A	NM_001783.3
CD79B	NM_000626.3
CD81	NM_004356.3
CD8A	NM_001768.6
CDC42	NM_001791.3
CDCA7	NM_031942.4
CEBPE	NM_001805.3
CFB	NM_001710.5
CFD	NM_001928.3
CFH*	NM_000186.3
CFI	NM_000204.4
CFP	NM_002621.2
CHD7	NM_017780.3
CIB1	NM_001277764.1
CIITA	NM_000246.3
CLCN7	NM_001287.5
CLPB	NM_030813.5
COL7A1	NM_000094.3
COPA	NM_004371.3
CORO1A*	NM_007074.3
CR2	NM_001006658.2
CSF2RA*	NM_006140.4
CSF2RB	NM_000395.2
CSF3R	NM_000760.3
CTC1	NM_025099.5
CTLA4	NM_005214.4
CTPS1	NM_001905.3
CTSC	NM_001814.5
CXCR2	NM_001557.3
CXCR4	NM_003467.2
CYBA	NM_000101.3
CYBB	NM_000397.3
CYP27A1	NM_000784.3
DBR1	NM_016216.3
DCLRE1C	NM_001033855.2
DDX58	NM_014314.3
DEF6	NM_022047.3
DGAT1	NM_012079.5

GENE	TRANSCRIPT
DIAPH1	NM_005219.4
DKC1	NM_001363.4
DNAJC21	NM_001012339.2
DNASE1L3	NM_004944.3
DNASE2	NM_001375.2
DNMT3B	NM_006892.3
DOCK2	NM_004946.2
DOCK8	NM_203447.3
DSG1	NM_001942.3
DTNBP1	NM_032122.4
DUOX2*	NM_014080.4
EFL1*	NM_024580.5
EIF2AK3	NM_004836.6
ELANE	NM_001972.2
EPG5	NM_020964.2
ERBIN	NM_001253697.1
ERCC2	NM_000400.3
ERCC3	NM_000122.1
ERCC6L2	NM_020207.4
EXTL3	NM_001440.3
FADD	NM_003824.3
FANCA	NM_000135.2
FANCB	NM_001018113.1
FANCE	NM_021922.2
FANCF	NM_022725.3
FANCI	NM_001113378.1
FANCL*	NM_018062.3
FAS	NM_000043.5
FASLG	NM_000639.2
FAT4	NM_024582.4
FCHO1	NM_001161357.1
FERMT1	NM_017671.4
FERMT3	NM_031471.5
FNIP1	NM_133372.2
FOXI3	NM_001135649.2
FOXN1	NM_003593.2
FOXP3	NM_014009.3
FPR1	NM_002029.3
G6PC	NM_000151.3
G6PC3	NM_138387.3
G6PD	NM_001042351.2
GATA1	NM_002049.3
GATA2	NM_032638.4
GFI1*	NM_005263.3
GINS1	NM_021067.4
GTF2E2	NM_002095.4
GTF2H5	NM_207118.2
GUCY2C	NM_004963.3
HAX1	NM_006118.3
HELLS	NM_018063.4
HMOX1	NM_002133.2
HPS1	NM_000195.4
HPS3	NM_032383.4
HPS4	NM_022081.5
HPS5	NM_181507.1
HPS6	NM_024747.5
HTRA2	NM_013247.4
HYOU1	NM_006389.4
ICOS	NM_012092.3
ICOSLG	NM_015259.5
IFIH1	NM_022168.3
IFNAR1	NM_000629.2
IFNAR2	NM_207585.2
IFNGR1	NM_000416.2
IFNGR2*	NM_005534.3
IGLL1	NM_020070.3
IKBKB	NM_001556.2
IKZF1	NM_006060.6
IL10	NM_000572.2
IL10RA	NM_001558.3
IL10RB	NM_000628.4
IL12B	NM_002187.2
IL12RB1	NM_005535.2
IL12RB2	NM_001559.2
IL17F	NM_052872.3
IL17RA	NM_014339.6
IL17RC	NM_153461.3
IL18BP	NM_173042.2
IL1RN	NM_173841.2
IL21	NM_021803.3
IL21R	NM_021798.3
IL23R	NM_144701.2
IL2RA	NM_000417.2
IL2RB	NM_000878.3
IL2RG	NM_000206.2
IL36RN	NM_012275.2
IL6R	NM_000565.3
IL6ST	NM_002184.3
IL7R	NM_002185.3
IRAK4	NM_016123.3
IRF2BP2	NM_182972.2
IRF4	NM_002460.3
IRF7	NM_004031.2
IRF8	NM_002163.2
IRF9	NM_006084.4
ISG15	NM_005101.3
ITCH	NM_031483.6
ITGAM	NM_000632.3
ITGB2	NM_000211.4
ITK	NM_005546.3
JAGN1	NM_032492.3
JAK1	NM_002227.3
JAK3	NM_000215.3
KAT6A	NM_006766.4
KDM6A*	NM_021140.3
KMT2A	NM_001197104.1
KMT2D	NM_003482.3
LAMTOR2	NM_014017.3
LAT	NM_001014987.1
LCK	NM_001042771.2
LCT	NM_002299.3
LIG1	NM_000234.2
LIG4	NM_002312.3
LIPA	NM_000235.3
LPIN2	NM_014646.2
LRBA	NM_006726.4
LRRC8A	NM_019594.3

GENE	TRANSCRIPT
LYN	NM_002350.3
LYST	NM_000081.3
MAD2L2	NM_001127325.1
MAGT1	NM_032121.5
MALT1	NM_006785.3
MAP3K14	NM_003954.4
MCM4	NM_005914.3
MEFV	NM_000243.2
MKL1	NM_020831.4
MOGS	NM_006302.2
MPLKIP	NM_138701.3
MS4A1	NM_152866.2
MSN	NM_002444.2
MTHFD1	NM_005956.3
MVK	NM_000431.3
MYD88	NM_002468.4
MYO5B	NM_001080467.2
MYSM1	NM_001085487.2
NBAS	NM_015909.3
NBN	NM_002485.4
NCF2	NM_000433.3
NCF4	NM_013416.3
NCKAP1L	NM_005337.4
NCSTN	NM_015331.2
NEUROG3	NM_020999.3
NFAT5	NM_138714.3
NFE2L2	NM_006164.4
NFKB1	NM_003998.3
NFKB2	NM_001077494.3
NFKBIA	NM_020529.2
NHEJ1	NM_024782.2
NHP2	NM_017838.3
NLRC4	NM_021209.4
NLRP1	NM_033004.3
NLRP12	NM_144687.3
NLRP3	NM_004895.4
NOD2	NM_022162.2
NOP10	NM_018648.3
NSMCE3	NM_138704.3

GENE	TRANSCRIPT
OAS1	NM_016816.3
ORAI1	NM_032790.3
OSTM1	NM_014028.3
OTULIN	NM_138348.4
PARN	NM_002582.3
PAX1	NM_006192.4
PEPD	NM_000285.3
PGM3	NM_001199917.1
PIK3CD	NM_005026.3
PIK3R1	NM_181523.2
PLCG2	NM_002661.4
PLVAP	NM_031310.2
PMM2	NM_000303.2
PNLIP	NM_000936.3
PNP	NM_000270.3
POLA1	NM_016937.3
POLD1*	NM_002691.3
POLD2	NM_006230.3
POLE	NM_006231.3
POLE2	NM_002692.3
POLR3A	NM_007055.3
POLR3F	NM_001282526.1
POMP	NM_015932.5
PRF1	NM_001083116.1
PRKCD	NM_006254.3
PRKDC	NM_006904.6
PSENEN	NM_172341.2
PSMA3	NM_002788.3
PSMB4	NM_002796.2
PSMB8	NM_148919.3
PSMG2	NM_020232.4
PSTPIP1	NM_003978.3
PTPRC*	NM_002838.4
RAB27A	NM_004580.4
RAC2	NM_002872.4
RAG1	NM_000448.2
RAG2	NM_000536.3
RANBP2*	NM_006267.4
RASGRP1	NM_005739.3

GENE	TRANSCRIPT
RBCK1	NM_031229.3
REL	NM_002908.3
RELA	NM_021975.3
RELB	NM_006509.3
RFWD3	NM_018124.3
RFX5	NM_000449.3
RFXANK	NM_003721.3
RFXAP	NM_000538.3
RHOH	NM_004310.4
RIPK1	NM_003804.4
RMRP	NR_003051.3
RNASEH2A	NM_006397.2
RNASEH2B	NM_024570.3
RNASEH2C	NM_032193.3
RNF113A	NM_006978.2
RNF168	NM_152617.3
RNF31	NM_017999.4
RNU4ATAC	NR_023343.1
RORC	NM_005060.3
RPSA	NM_002295.5
RTEL1	NM_001283009.1
SAMD9	NM_017654.3
SAMD9L	NM_152703.4
SAMHD1	NM_015474.3
SAR1B*	NM_001033503.2
SCO2	NM_005138.2
SEC61A1	NM_013336.3
SEMA3E	NM_012431.2
SERPING1	NM_000062.2
SGPL1	NM_003901.3
SH2D1A	NM_002351.4
SH3BP2	NM_003023.4
SH3KBP1	NM_031892.2
SI*	NM_001041.3
SIAE	NM_170601.4
SKIV2L	NM_006929.4
SLC10A2	NM_000452.2
SLC26A3	NM_000111.2
SLC29A3	NM_018344.5

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GENE	TRANSCRIPT
SLC35C1	NM_018389.4
SLC37A4	NM_001164277.1
SLC39A7	NM_001077516.1
SLC46A1	NM_080669.5
SLC51B	NM_178859.3
SLC5A1	NM_000343.3
SLC7A7	NM_001126106.2
SLC9A3*	NM_004174.3
SLX4	NM_032444.2
SMARCAL1	NM_014140.3
SMARCD2	NM_001098426.1
SNX10	NM_001199835.1
SP110	NM_004509.3
SPINK5	NM_006846.3
SPINT2	NM_021102.3
SPPL2A	NM_032802.3
SRP54	NM_003136.3
SRP72	NM_006947.3
STAT1	NM_007315.3
STAT2	NM_005419.3
STAT3	NM_139276.2
STAT4	NM_003151.3
STAT5B*	NM_012448.3
STIM1	NM_003156.3
STK4	NM_006282.3
STN1	NM_024928.4
STX11	NM_003764.3
STX3	NM_004177.4
STXBP2	NM_006949.3
TAOK2	NM_016151.3
TAP1	NM_000593.5
TAP2	NM_000544.3
TAPBP	NM_003190.4
TAZ	NM_000116.4
TBX1	NM_080647.1
TCF3	NM_003200.4;NM_001136139.3
TCIRG1	NM_006019.3
TCN2	NM_000355.3
TERC	NR_001566.1

GENE	TRANSCRIPT
TERT	NM_198253.2
TFRC	NM_003234.3
TGFB1	NM_000660.5
TGFBR1	NM_004612.2
TGFBR2	NM_003242.5
THBD	NM_000361.2
TICAM1	NM_182919.3
TIMM50	NM_001001563.3
TINF2	NM_001099274.1
TLR3	NM_003265.2
TLR7	NM_016562.3
TMC6	NM_007267.7
TMC8	NM_152468.4
TMEM173	NM_198282.3
TMPRSS15	NM_002772.2
TNFAIP3	NM_006290.3
TNFRSF11A	NM_003839.3
TNFRSF13B	NM_012452.2
TNFRSF13C	NM_052945.3
TNFRSF1A	NM_001065.3
TNFRSF4	NM_003327.3
TNFRSF6B	NM_003823.3
TNFRSF9	NM_001561.5
TNFSF11	NM_003701.3
TNFSF12	NM_003809.2
TONSL	NM_013432.4
TOP2B*	NM_001068.3
TP63	NM_003722.4
TPP2	NM_003291.2
TRAF3	NM_003300.3
TRAF3IP2	NM_147686.3
TREX1	NM_033629.4
TRNT1	NM_182916.2
TTC37	NM_014639.3
TTC7A	NM_020458.3
TYK2	NM_003331.4
UNC13D	NM_199242.2
UNC45A	NM_018671.4
UNC93B1*	NM_030930.3

GENE	TRANSCRIPT
UNG	NM_080911.2
USB1	NM_024598.3
VAV1	NM_005428.3
VPS13B	NM_017890.4
VPS45	NM_007259.4
WAS	NM_000377.2
WDR1	NM_017491.3
WIPF1	NM_001077269.1
WNT2B	NM_024494.2
WRAP53	NM_018081.2
XIAP	NM_001167.3
ZAP70	NM_001079.3
ZBTB24	NM_014797.2
ZCCHC8	NM_017612.4
ZNF341	NM_032819.4

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, 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 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. 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. DUOX2: Deletion/duplication and sequencing analysis is not offered for exons 6-7. SAR1B: Deletion/duplication analysis is not offered for exon 5. TOP2B: Deletion/duplication analysis is not offered for exon 5. POLD1: Sequencing analysis for exons 22 includes only cds +/- 10 bp. CORO1A: Deletion/duplication and sequencing analysis is not offered for exon 11. GFI1: Sequencing analysis for exons 6 includes only cds +/- 0 bp. CFH: Deletion/duplication analysis is not offered for exons 20, 22 and sequencing analysis is not offered for exons 15, 20, 22. KDM6A: Sequencing analysis for exons 18 includes only cds +/- 10 bp. PTPRC: Sequencing analysis is not offered for exons 3, 15. ADGRE2: Deletion/duplication analysis is not offered for exons 3, 6-9 and sequencing analysis is not offered for exons 6-9. Sequencing analysis for exons 17 includes only cds +/- 10 bp. SI: Deletion/duplication analysis is not offered for exon 7. EFL1: Deletion/duplication and sequencing analysis is not offered for exons 7, 15. ALPK1: Sequencing analysis for exons 8 includes only cds +/- 10 bp. RANBP2: Deletion/duplication and sequencing analysis is not offered for exons 1-11, 15-29. AK2: Deletion/duplication and sequencing analysis is not offered for exon 6. SLC9A3: Deletion/duplication analysis is not offered for exon 8. STAT5B: Deletion/duplication and sequencing analysis is not offered for exons 6-8. ATM: Sequencing analysis for exons 6, 24, 43 includes only cds +/- 10 bp. UNC93B1: Deletion/duplication analysis is not offered for exon 11. FANCL: Sequencing analysis for exons 4, 10 includes only cds +/- 10 bp. CSF2RA: Deletion/duplication analysis is not offered for this gene. IFNGR2: Sequencing analysis for exons 6 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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Clinical Molecular 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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