**REPORT DATE:** 24/01/2025

**LAB #:** IM365 / 25IG001626

**NAME:**

**HKID:**

**DATE OF BIRTH:**

**SEX/AGE:**

**ETHNICITY:** Unknown

**SPECIMEN COLLECTED:** 22/01/2025

**SPECIMEN ARRIVED:**  22/01/2025

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**SPECIMEN:**

EDTA Blood

**CLINICAL HISTORY:**

Allergic rhinitis. With recurrent sinusitis, nasopharyngeal mass with pseudomonas aeruginosa septicemia.

**TYPE OF TESTING REQUESTED:**

Diagnostic confirmation

**TEST DESCRIPTION:**

In-house Immunological Disorders SuperPanel gene panel from WES was tested by next generation sequencing, and 516 genes were included in the panel test.

**SUMMARY OF RESULT(S):**

**One likely pathogenic variant and one VUS were detected in the IRAK4 gene.**

**One likely pathogenic variant was detected in the TNFRSF13B gene.**

**Results :**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene name/OMIM** | **Transcript/**  **Variant in HGVS Nomenclature** | **Exon Location** | **Genotype/**  **Zygosity** | **Inheritance** | **Parent origin** | **Classification** |
| **IRAK4**  **\*606883** | NM\_016123.4:c.1119delT  NP\_057207.2:p.Phe373Leufs\*6 | 9 | Heterozygous | AR | N/A | Likely Pathogenic |
|  | **Position REF/ALT** | **Assembly** | **SNP Identifier** | **Phenotype** | | |
|  | 12:43782482 T/- | GRCh38 | rs1376102243 | Immunodeficiency 67 # 607676 | | |
| **Gene name/OMIM** | **Transcript/**  **Variant in HGVS Nomenclature** | **Exon Location** | **Genotype/**  **Zygosity** | **Inheritance** | **Parent origin** | **Classification** |
| **IRAK4**  **\*606883** | NM\_016123.4:c.65T>C  NP\_057207.2:p.Leu22Pro | 2 | Heterozygous | AR | N/A | VUS |
|  | **Position REF/ALT** | **Assembly** | **SNP Identifier** | **Phenotype** | | |
|  | 12:43768176 T/C | GRCh38 | N/A | Immunodeficiency 67 # 607676 | | |
| **Gene name/OMIM** | **Transcript/**  **Variant in HGVS Nomenclature** | **Exon Location** | **Genotype/**  **Zygosity** | **Inheritance** | **Parent origin** | **Classification** |
| **TNFRSF13B**  **\* 604907** | NM\_012452.3:c.303\_306delinsTTG  NP\_036584.1: p.(Tyr102Cysfs\*11) | 3 | Heterozygous | AD/AR | N/A | Likely Pathogenic |
|  | **Position REF/ALT** | **Assembly** | **SNP Identifier** | **Phenotype** | | |
|  | 17:16948877 GTAT/CAA  -17:16948880 | GRCh38 | N/A | Immunodeficiency, common variable, 2  # 240500  Immunoglobulin A deficiency 2 # 609529 | | |

**INTERPRETATION / RECOMMENDED ACTION:**

One likely pathogenic variant and one VUS were detected in the IRAK4 gene. IRAK4 variants have been associated with autosomal recessive immunodeficiency condition, that predisposed patients to recurrent pyogenic bacterial infections. Hence, the findings may support possible autosomal recessive condition involving the IRAK4 gene, if the variants were in trans. Clinical correlations and testing of parental samples would provide useful information in the assessment of the variants, such as the phasing, and is recommended. Genetic counseling by a qualified healthcare professional with molecular genetics training is suggested for further follow-ups.

In addition, one likely pathogenic variant was detected in the TNFRSF13B gene. Monoallelic or biallelic mutations in TNFRSF13B are associated with a form of Common Variable Immunodeficiency (CVID) [OMIM: 240500] as well as selective IgA deficiency [OMIM: 609529]. Incomplete penetrance has been observed in patients carrying monoallelic TNFRSF13B mutation, with some carriers being asymptomatic. CVID is a clinically and genetically heterogeneous entity, primarily presenting as an antibody deficiency. In addition, non-infectious complications linked to underlying immune dysregulation are present in a subset of patients, such as granulomatous complications, autoimmunity and lymphoproliferations.

Please note that variants assessment and interpretations may change with time when additional information from the patient’s assessment or in the literature is available in the future. Test results should always be interpreted with clinical context, family history and other relevant data. Further investigations may be considered if clinically indicated.

**COMMENTS:**

IRAK4 encodes a kinase that activates NF-kappaB in both the Toll-like receptor (TLR) and T-cell receptor (TCR) signalling pathways. The protein is essential for most innate immune responses**.** IRAK4 deficiency is an autosomal recessive immunodeficiency condition.Blood leukocytes derived from IRAK-4-deficient patients display impaired responses to most of the TLR and IL-1R agonists tested. Available clinical data in the literature with IRAK-4 deficiencies suggest instead a narrow susceptibility to invasive bacterial infections, mostly caused by gram-positive bacteria, such as Streptococcus pneumoniae and Staphylococcus aureus in particular, with rare infections caused by gram-negative bacteria, such as Pseudomonas aeruginosa and Shigella sonnei (PMID 21057262). Though IRAK-4 deficiencies classically confer a predisposition to severe bacterial infection with impairment of the ability to increase plasma CRP concentrations and mount fever, patients with IRAK-4 and invasive bacterial infection may also present with high temperature and high levels of CRP, total leukocytes, and neutrophils (PMID 21057262).

The patient is heterozygous for a likely pathogenic variant, NM\_016123.4:c.1119delT, and a VUS NM\_016123.4:c.65T>C in the IRAK4 gene. Since parental samples were not available at the time of analysis, the phasing of these two variants is uncertain. The NM\_016123.4:c.1119delT variation generates a 'Frameshift' as coding effect, and is expected to undergo nonsense mediated decay and disruption of gene function. The variant is rare and present in extremely low frequency in the population database (gnomAD exome 0.0007% AF), without the presence of homozygotes. The variant was reported in dbSNP (rs1376102243). Overall, the variant NM\_016123.4:c.1119delT is classified as likely pathogenic. The patient is also heterozygous for a missense variant, NM\_016123.4:c.65T>C, in the IRAK4 gene. The missense mutation leads to the change of leucine to proline at codon 22, which is highly conserved in orthologues studies, though there is moderate physicochemical difference between the two amino acids. In silico computational tools predict deleterious impact of the variant (REVEL 0.88). The variant is rare and is absent in population databases. It has not been reported in affected individual. With limited existing information, overall, the variant NM\_016123.4:c.65T>C was classified as VUS.

TNFRSF13B encodes TACI (transmembrane activator and CAML interactor), a transmembrane protein of the TNF receptor superfamily found predominantly on the surface of B cells. TACI mediates activation of transcription factors NF-AT, NF-kappa-B, and AP-1, and is involved in the stimulation of B- and T-cell function and the regulation of humoral immunity. TNFRSF13B was first reported in relation to autosomal recessive common variable immunodeficiency 2 (CVID 2) in 2001 (PMID: 16007087 and PMID: 16007086). CVID is a clinically and genetically heterogeneous entity, primarily presenting as an antibody deficiency with low levels of serum IgG, IgA, and/or IgM, functional antibody deficiency, and recurrent sinopulmonary bacterial infections. In addition, non-infectious complications linked to underlying immune dysregulation are present in a subset of patients, such as granulomatous complications, autoimmunity and lymphoproliferations. Of note, variants in TNFRSF13B have been observed in CVID patients in both the monoallelic and biallelic forms. Heterozygous mutations in TNFRSF13B may lead to impaired ligand binding and contribute to defects in cell activation and B cell tolerance, but the connection between heterozygous mutations and disease is not clear, because normal relatives may have the same mutations without evidence of immune deficiency. In view of incomplete penetrance and can be found in many asymptomatic carriers, monoallelic variants may be considered risk alleles. On the other hand, heterozygous TACI variants do affect B cell activation and peripheral tolerance, which may predispose to autoimmunity (PMID: 24051380).

The patient is heterozygous for a variant NM\_012452.3:c.303\_306delinsTTG, that generate a frameshift coding effect. The frameshift starts at codon Tyr102, and the new reading frame ends in a STOP codon at position 11 downstream. The frameshift variant is expected to lead to nonsense mediated decay and assumed to disrupt gene function. The variant is rare is absent in population databases. However, the variant was reported and classified as likely pathogenic in ClinVar (Variant ID 2633419). Overall, the variant is classified as likely pathogenic.

**VARIANT CLASSIFICATION:**

Following the ACMG/AMP criteria for pathogenicity categorization, the variant NM\_016123.4:c.1119delT has been classified as likely pathogenic as it has the following characteristics:

PVS1: null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease

PM2 \_support: Absent from controls (or at extremely low frequency if recessive) in Genome Aggregation Database

Following the ACMG/AMP criteria for pathogenicity categorization, the variant NM\_016123.4:c.65T>C has been classified as VUS as it has the following characteristics:

PP3 \_moderate: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) (REVEL 0.88)

PM2 \_support: Absent from controls (or at extremely low frequency if recessive) in Genome Aggregation Database

Following the ACMG/AMP criteria for pathogenicity categorization, the variant NM\_012452.3:c.303\_306delinsTTG has been classified as likely pathogenic as it has the following characteristics:

PVS1: null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease

PM2 \_support: Absent from controls (or at extremely low frequency if recessive) in Genome Aggregation Database

**APPENDIX:**

**SEQUENCING PERFORMANCE METRICS**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PANELS** | **GENES** | **EXONS/REGIONS** | **BASES** | **% of target region >20X** | **Uniformity (%)** | **MEDIAN COVERAGE** |
| Immunological Disorders SuperPanel | 516 | 15,072 | 2,229,416 | 99.85 | 99.63 | 217X |

**TARGET REGION AND GENE LIST**

ACD, ACP5, ACTB\*, ADA, ADA2, ADAM17, ADAMTS13, ADAR, AICDA#, AIRE#, AK2, ALPI, ALPK1, ANGPT1, ANKZF1, AP1S3, AP3B1, AP3D1, APOL1, ARHGEF1, ARPC1B, ARPC5, ATM, ATP6AP1#, B2M, BACH2, BCL10, BCL11B#, BLK, BLM, BLNK, BLOC1S6, BTK#, C1QA, C1QB, C1QC, C1R, C1S, C2\*#, C2orf69#, C3#, C5, C6, C7, C8A, C8B, C8G, C9, CARD10#, CARD11, CARD14#, CARD8, CARD9, CARMIL2, CASP10, CASP8, CBLB, CCBE1#, CCDC103, CCDC39, CCDC40#, CCDC65, CCNO, CD19, CD247, CD27, CD28, CD3D, CD3E, CD3G, CD4, CD40, CD40LG#, CD46\*, CD55, CD59, CD70, CD79A, CD79B, CD81, CD8A, CDC42, CDCA7, CEBPE, CFAP298, CFAP300, CFB, CFD#, CFH\*, CFI#, CFP#, CFTR, CHD7, CHUK, CIB1, CIITA, CLEC7A, CLPB#, COL7A1#, COPA, COPG1, CORO1A\*#, CR2, CRACR2A, CSF2RA, CSF2RB, CSF3R, CTC1#, CTLA4, CTNNBL1, CTPS1, CTSC, CXCR2, CXCR4, CYBA, CYBB#, CYBC1, DBR1, DCLRE1B, DCLRE1C\*, DDX58, DEF6, DGKE, DIAPH1, DKC1, DNAAF11, DNAAF2, DNAAF3#, DNAAF4, DNAAF5, DNAAF6, DNAH1#, DNAH11\*#, DNAH5, DNAH9, DNAI1, DNAI2, DNAJB13, DNAJC21, DNAL1, DNASE1, DNASE1L3, DNASE2#, DNMT3B, DOCK11#, DOCK2, DOCK8, DPP9, DSG1, DTNBP1#, EFL1#, ELANE, EPG5, ERBIN, ERCC2, ERCC3, ERCC6L2, EXTL3, F12, FAAP24, FADD, FANCA, FANCB, FANCE, FANCF, FANCI#, FANCL, FAS, FASLG, FAT4, FCGR3A#, FCHO1, FCN3, FERMT1, FERMT3, FGL2, FNIP1, FOXN1#, FOXP3#, FPR1, G6PC3, G6PD#, GAS8#, GATA1#, GATA2, GFI1, GIMAP6, GINS1, GTF2H5, HAVCR2, HAX1#, HCK, HELLS#, HMOX1, HPS1\*, HPS3, HPS4, HPS5, HPS6, HS3ST6, HTRA2, HYOU1#, ICOS, ICOSLG#, IFIH1#, IFNAR1, IFNAR2, IFNG, IFNGR1, IFNGR2, IGHM, IGLL1, IKBKB, IKZF1, IKZF2, IKZF3, IL10, IL10RA, IL10RB#, IL12B, IL12RB1, IL12RB2, IL17F, IL17RA, IL17RC, IL18BP, IL1RN, IL21, IL21R, IL23R, IL2RA, IL2RB, IL2RG#, IL36RN, IL37, IL6R#, IL6ST, IL7, IL7R, INO80, IPO8, IRAK1#, IRAK4, IRF2BP2, IRF3, IRF4, IRF7, IRF8#, IRF9, ISG15, ITCH, ITGB2#, ITK, ITPKB, IVNS1ABP, JAGN1, JAK1, JAK3, KDM6A#, KMT2A, KMT2D#, KNG1, KRAS, LACC1, LAMTOR2, LCK, LCP2, LIG1, LIG4, LPIN2, LRBA, LRRC56, LRRC8A, LY96, LYN, LYST#, MAD2L2, MAGT1#, MALT1#, MAN2B2#, MAP3K14, MAPK8, MASP2, MCIDAS, MCM10#, MCM4, MEFV, MOGS, MPEG1, MRE11, MRTFA, MS4A1, MSH6, MSN\*#, MTHFD1, MVK, MYD88, MYOF, MYSM1, NBAS, NBN, NCF2, NCF4, NCKAP1L, NCSTN, NFAT5, NFE2L2, NFKB1, NFKB2, NFKBIA, NHEJ1, NHP2, NLRC4, NLRP1, NLRP12#, NLRP3, NOD2, NOP10, NOS2#, NRAS, NSMCE3, OAS1, ODAD1#, ODAD3#, OFD1#, ORAI1, OTULIN, PARN\*, PAX1, PAX5, PDCD1, PEPD, PGM3, PIK3CD\*, PIK3CG, PIK3R1, PLCG2, PLG#, PLVAP, PMM2, PMS2\*#, PNP, POLA1#, POLD1, POLD2, POLD3, POLE, POLE2, POLR3A, POLR3C, POLR3F, POMP, POU2AF1, PRF1, PRKCD, PRKDC#, PSENEN, PSMA3, PSMB10, PSMB4, PSMB8, PSMB9, PSMG2, PSTPIP1, PTEN\*, PTPN2, PTPRC, RAB27A, RAC2, RAG1, RAG2, RANBP2, RASGRP1, RBCK1#, RC3H1, REL, RELA, RELB, RFWD3, RFX5, RFXANK, RFXAP, RGS10, RHBDF2, RHOH, RIPK1, RMRP, RNASEH2A, RNASEH2B, RNASEH2C, RNF113A, RNF168, RNF31, RNU4ATAC, RORC, RPGR#, RPSA#, RSPH1, RSPH3, RSPH4A, RSPH9, RTEL1, SAMD9, SAMD9L, SAMHD1, SASH3#, SBDS, SEC61A1, SEMA3E, SERPING1, SGPL1, SH2D1A#, SH3BP2, SKIV2L, SLC29A3#, SLC35C1, SLC37A4#, SLC39A7#, SLC46A1, SLC7A7, SLC9A3, SLX4#, SMARCAL1#, SMARCD2, SNORA31, SOCS1, SP110, SPAG1, SPI1, SPINK5, SPPL2A, SRP19, SRP54, SRP72\*, SRPRA, STAT1, STAT2, STAT3, STAT4, STAT5B\*#, STAT6, STIM1, STING1, STK4, STN1, STX11, STXBP2#, STXBP3, SYK, TAFAZZIN#, TAOK2#, TAP1, TAP2, TAPBP, TBCE, TBK1, TBX1, TBX21, TCF3, TCN2, TERC, TERT, TET2, TFRC#, TGFB1, TGFBR1, TGFBR2, THBD, TICAM1, TINF2, TIRAP, TLR3, TLR7#, TLR8, TMC6, TMC8, TNFAIP3#, TNFRSF13B, TNFRSF13C, TNFRSF1A, TNFRSF4, TNFRSF9, TNFSF12, TNFSF13, TOM1, TOP2B, TPP1, TPP2, TRAC, TRAF3, TRAF3IP2, TREX1, TRIM22, TRIM69, TRNT1, TTC37, TTC7A, TYK2#, UBA1#, UNC119, UNC13D, UNC93B1\*#, UNG, USB1, VPS13B, VPS45, WAS#, WDR1, WIPF1, WRAP53#, XIAP\*#, ZAP70, ZBTB24, ZCCHC8, ZMYND10, ZNF341\*, ZNFX1

\*Some, or all, of the gene is duplicated in the genome. #The gene has suboptimal coverage when >90% of the gene’s target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. The sensitivity to detect variants may be limited in genes marked with an asterisk (\*) or number sign (#).

This panel targets protein coding exons, exon-intron boundaries (± 30 bps). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs). This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize copy number variant, balanced translocations or complex inversions, and it may not detect low-level mosaicism.

**METHODS:**

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using silica-membrane-based method. DNA quality and quantity were assessed using spectrophotometric and fluorometric method. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented by fragmentation enzymes. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (36.5 Mb of human protein coding regions with additional clinically relevant non-coding pathogenic and likely pathogenic variants) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing by synthesis (SBS) method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the output.

Bioinformatics and quality control: Illumina DRAGEN Bio-IT Platform onboard (NextSeq2000) pipeline (DRAGEN Enrichment v3.10.12) was used for secondary analysis of NGS data, includes data decompressing, mapping, aligning, sorting, duplicate marking & removing, and variant calling. In brief, base called raw sequencing data was transformed into FASTQ format and sequence reads of each sample were mapped to the human reference genome (GRCh38). Read alignment, duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using DRAGEN DNA algorithms. Variant data (VCF output files) was annotated using Golden Helix VarSeq software v2.5.0 with a variety of public variant databases including but not limited to gnomAD 3.1.2v2, ClinVar, OMIM, and CADD score v1.6, and Mastermind. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up.

Interpretation: Laboratory immunologists/scientific officers assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to 2015 ACMG classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported. Moreover, variants in genes that is not related to the patient’s clinical phenotype at the time of analysis or with low post test probability VUS may not be reported. For pathogenic/ likely pathogenic variant(s) within the gene panel that are unrelated to the request indication will be included in the appendix section as incidental findings. Interpretation of the test results is limited by the information that is currently available. Better interpretation should be possible in the future as more data and knowledge about human genetics and this specific disorder are accumulated. It is possible that the variant classification, or gene-disease association may change with time. The laboratory would not reinterpret or reissue reports automatically. However, a request for reinterpretation may be considered in the form of new request.

Variant classification: Our variant classification follows the ACMG guideline 2015, and the ClinGen SVI recommendations. ACGS 2020 Best Practice Guideline was also referenced for variant classification and interpretation.

Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual.

Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call.

Analytic validation: The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.42%, indels 1-50 bps 99.26%, and specificity >99.9% for both SNVs and indels). It does not detect very low level mosaicism.

Assay limitations: Please note that the overall coverage of each genes varies and each individual may have slightly different coverage yield. This test does not detect the following: copy number variation, complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±30 base pairs from exon-intron boundary unless otherwise indicated. Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The study does not cover assessment in repeat expansions, mutations involved in tri-allelic inheritance, mitochondrial genome variants, epigenetic effects, oligogenic. The current platform is for germline variant assessment, and that somatic variants may not be detected. Laboratory error is also possible.

Further test details could be found at Division’s webpage: https://hkwc.home/webapps/Dept/CIMM/CellFnMolecularImmLab.aspx

**DISCLAIMERS:**

(1) The above results refer only to the sample received.

(2) The design of the virtual gene panel is based on the reference gene list published by the International Union of Immunological Societies (IUSI) expert committee: Journal of clinical immunology vol. 40,1 (2020): 24-64, and the Panel App Australia (https://panelapp.agha.umccr.org/)

(3) Do NOT copy this report without permission of the laboratory. Further information is available on request. This report must be read in its entirety.

(4) There are possible sources of error for any DNA studies. Errors can result from trace contamination, sample mix up, erroneous paternity identification, rare technical errors, clerical errors and rare genetic variants that interfere with analysis etc.

(5) A negative result does not rule out the possibility that the tested individual carries a rare unexamined variant/gene(s) or variant in the undetectable region. The clinical sensitivity of the test may vary widely depending on the clinical and family history. Please also refers to assay limitations for details

(6) This laboratory-developed test has been independently validated by the Clinical Immunology Division (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration.

**Reported By:**

**Dr. Edmund Tung**

**Signed Out By:**

**Consultant Immunologist**

**Dr. Au Yuen Ling Elaine**

\*\*\*\*\*\*\*\*\*\* End of report \*\*\*\*\*\*\*\*\*\*