**REPORT DATE: 07/01/2025**

**LAB #:** IM456 / 25IG001602

**NAME:**

**HKID:**

**DATE OF BIRTH:**

**SEX/AGE:**

**ETHNICITY:**

**SPECIMEN COLLECTED:** 03/01/2025

**SPECIMEN ARRIVED:**  03/01/2025

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**SPECIMEN:**

EDTA Blood

**CLINICAL HISTORY:**

Dermatomyositis

**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. Trio analysis has been performed.

**SUMMARY OF RESULT(S):**

**No disease-causing variant detected to fully account for the patient’s phenotype. However, details on some additional findings have been included for reference.**

**ADDITIONAL FINDINGS:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene name/OMIM** | **Transcript/**  **Variant in HGVS Nomenclature** | **Exon Location** | **Genotype/Zygosity** | **Inheritance** | **Parent origin** | **Classification** |
| **BLM**  **\* 604610** | NM\_000057.4:c.3864G>A  NP\_000048.1:p.Trp1288Ter | 20/22 | Heterozygous | Autosomal Recessive | Maternal | Likely Pathogenic |
|  | **Position REF/ALT** | **Assembly** | **SNP Identifier** | **Phenotype** | | |
|  | 15:90809249 G/A | GRCh38 | rs2151198391 | Bloom syndrome  MIM#210900 | | |

**INTERPRETATION / RECOMMENDED ACTION:**

Sequence analysis in current study with the Immunology gene panel did not detect any known disease-causing variants that could fully account the patient’s phenotype as described to the laboratory at the time of interpretation. Please note that negative results do not rule out the diagnosis of a genetic disorder since some of the DNA abnormalities may be undetectable by the current applied technology. Further investigations may be considered if clinically indicated.

A heterozygous maternal inherited likely pathogenic variant in the BLM gene was detected, that the clinical relevance is uncertain. Please note that these findings do NOT confirm a genetic diagnosis. The diagnosis of this case would rely on clinical judgement/criteria. Please refer to specialists for assessment and further follow-ups.

Please note that the finding of a single monoallelic likely pathogenic variant in an autosomal recessive condition may mean either an incidental finding of carrier status, or a second variant inherited in trans but not detected by current platform due to the assay limitation. Please also 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, such as whole exome/ genome sequencing, assays targeting somatic mutation, CNV etc, may be considered if clinically indicated.

**COMMENTS:**

The BLM gene encodes the RECQL3 protein, a member of a protein family called RecQ helicases. Helicases are enzymes that bind to DNA and unwind the double helix so that DNA replication or DNA repair can take place. The BLM protein helps to prevent excess sister chromatid exchanges and is also involved in other processes that help maintain the stability of the DNA during the replication process. BLM gene variation is associated with an autosomal recessive condition, Bloom syndrome, which is a rare disorder characterized by short stature, a skin rash that develops after sun exposure, and an increased risk of cancer. Without the BLM protein, the cell is less able to repair DNA damage caused by ultraviolet light, which results in increased sun sensitivity. Genetic changes that allow cells to divide in an uncontrolled way predispose to the cancers that occur in people with Bloom syndrome. Previous publication has shown that BLM-deficient fibroblasts show constitutive up-regulation of inflammatory interferon-stimulated gene (ISG) expression, which is mediated by the cGAS–STING–IRF3 cytosolic DNA–sensing pathway. It has also been shown that BS patients demonstrate elevated ISG expression in peripheral blood, suggesting that BLM keeps inflammatory gene induction resulting from DNA damage in check (PMID: 30936263).

The patient is heterozygous for a maternal inherited likely pathogenic variant in the BLM gene. The variant is a nonsense mutation, changing the codon from TGG to TGA, leading to a premature stop at codon 1288. The variant is rare and is absent in population database, such as GnomAd, but was reported in dbSNP (rs2151198391). The variant was classified as pathogenic in ClinVar (Variation ID: 1456846). Overall, the variant is classified as likely pathogenic by ACMG classification.

**VARIANT CLASSIFICATION:**

Following the ACMG/AMP criteria for pathogenicity categorization, the variant NM\_000057.4:c.3864G>A 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 \_supp: Absent from controls (or at extremely low frequency if recessive) in Genome Aggregation Database

**SEQUENCING PERFORMANCE METRICS**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PANELS** | **GENES** | **EXONS/REGIONS** | **BASES** | **% of target region >20X** | **Uniformity (%)** | **MEDIAN COVERAGE** |
| Immunological Disorders SuperPanel | 516 | 15,072 | 2,229,416 | 99.68 | 99.47 | 176X |

**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, dbNSFP functional predictions and Scores v4.2 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 may not be reported. 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 (please see the list of non-coding variants covered by the test). 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 \*\*\*\*\*\*\*\*\*\*