

## Whole Exome Sequencing Analysis – Carrier Screening

<b>Patient name</b>	<b>: Mr. XXX</b>	<b>Mrs. YYY</b>
<b>Gender/ Age</b>	<b>: Male/ 43 years</b>	<b>Female/ 34 years</b>
<b>PIN</b>	<b>: XX</b>	<b>XX</b>
<b>Sample no</b>	<b>: XX</b>	<b>XX</b>
<b>Specimen</b>	<b>: Peripheral blood</b>	<b>Peripheral blood</b>
<b>Sample collection date</b>	<b>: XX</b>	<b>XX</b>
<b>Sample receipt date</b>	<b>: XX</b>	<b>XX</b>
<b>Report date</b>	<b>: XX</b>	<b>XX</b>
<b>Hospital/Clinic</b>	<b>: XX</b>	

### Clinical history

Mr. XXX and Mrs. YYY are third-degree consanguineous couple and presented with primary infertility. They have a history of six failed IUI cycles. Mrs. YYY has a history of PCO and Mr. XXX semen analysis indicative of normozoospermia. Both partners karyotype revealed normal chromosome complement. He was born to 3rd degree consanguineous parents. Mr. XXX and Mrs. YYY has been evaluated for pathogenic variations.

### Results

Mr. XXX is found to be carrier of likely pathogenic variants in the *EARS2* gene (p.Val218SerfsTer2) and *DYM* gene (p.Tyr496Ter).

Mrs. YYY is found to be carrier of likely pathogenic variants in the *SLC12A1* gene (p.Ala508Thr) and *PKHD1L1* gene (c.6176-1G>C).

## List of significant carrier variants identified:

Disease	Mr. XXX	Mrs. YYY
Combined oxidative phosphorylation deficiency 12 <b>(OMIM#614924)</b>  <b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/1070</b> <b>Genetic Prevalence: 1/4578748</b>	<b>CARRIER</b> Gene: <i>EARS2</i> Exon 4, c.652_655delGTCA p.Val218SerfsTer2 Heterozygous  <b>Classification: Likely Pathogenic</b>	<b>NON - CARRIER</b>
Dyggve-Melchior-Clausen disease <b>(OMIM#223800)</b>  Smith-McCort dysplasia <b>(OMIM#607326)</b>  <b>Mode of inheritance: AR</b>	<b>CARRIER</b> Gene: <i>DYM</i> Exon 14, c.1488T>A p.Tyr496Ter Heterozygous  <b>Classification: Likely Pathogenic</b>	<b>NON - CARRIER</b>
Bartter syndrome, type 1 <b>(OMIM#601678 )</b>  <b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/666</b> <b>Genetic Prevalence: 1/1771049</b>	<b>NON - CARRIER</b>	<b>CARRIER</b> Gene: <i>SLC12A1</i> Exon 12, c.1522G>A p.Ala508Thr Heterozygous  <b>Classification: Likely Pathogenic</b>
Deafness, autosomal recessive 124 <b>(OMIM#620794)</b>  <b>Mode of inheritance: AR</b>	<b>NON - CARRIER</b>	<b>CARRIER</b> Gene: <i>PKHD1L1</i> Intron 40, c.6176-1G>C 3' Splice site Heterozygous  <b>Classification: Likely Pathogenic</b>

\*Genetic test results are reported based on the recommendation of American College of Medical Genetics [1].  
No other variant that warrants to be reported for the given clinical indication was identified.

## Variant Interpretation

### Interpretation for the significant carrier variants identified in Mr.XXX

#### **EARS2: c.652\_655delGTCA**

**Variant summary:** A heterozygous four base pair deletion in exon 4 of the *EARS2* gene (chr16:g.23535191delTGAC, NM\_001083614.2, Depth: 117x) that results in a frameshift and premature truncation of the protein 2 amino acids downstream to codon 218 (p.Val218SerfsTer2) was detected. This

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variant is a frameshift variant which occurs in an exon of *EARS2* upstream where nonsense mediated decay is predicted to occur. This variant is predicted to cause loss of normal protein function through protein truncation.

**Population frequency:** This variant has not been reported in gnomAD database and 1000 genomes database.

***In-silico* predictions:** The *in-silico* predictions of the variant are damaging by MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

**OMIM phenotype:** Combined oxidative phosphorylation deficiency 12 (OMIM#614924) is caused by homozygous or compound heterozygous mutation in the *EARS2* gene (OMIM\*612799). This disease follows autosomal recessive pattern of inheritance [2].

**Variant classification:** Based on the evidence, this variant is classified as a likely pathogenic variant.

#### ***DYM: c.1488T>A***

**Variant summary:** A heterozygous stop gained variation in exon 14 of the *DYM* gene (chr18:g.49209688A>T, NM\_001353214.3, Depth: 150x) that results in the premature truncation of the protein at codon 496 (p.Tyr496Ter) was detected. This variant is a stop gained variant which occurs in an exon of *DYM* upstream where nonsense mediated decay is predicted to occur. This variant is predicted to cause loss of normal protein function through protein truncation.

**Population frequency:** This variant has minor allele frequency of 0.0007% in gnomAD database and has not been reported in 1000 genomes databases.

***In-silico* prediction:** The reference codon is conserved across mammals in PhyloP and GERP++ tools.

**OMIM phenotype:** Dyggve-Melchior-Clausen disease (OMIM#223800) and Smith-McCort dysplasia (OMIM#607326) are caused by homozygous or compound heterozygous mutation in the *DYM* gene (OMIM\*607461). These diseases follow autosomal recessive pattern of inheritance [2].

**Variant classification:** Based on the evidence, this variant is classified as a likely pathogenic variant.

### **Interpretation for the significant carrier variants identified in Mrs.YYY**

#### ***SLC12A1: c.1522G>A***

**Variant summary:** A heterozygous missense variation in exon 12 of the *SLC12A1* gene (chr15:g.48246978G>A, NM\_000338.3, Depth: 251x) that results in the amino acid substitution of Alanine for Threonine at codon 508 (p.Ala508Thr) was detected.

**Population frequency:** This variant has minor allele frequency of 0.0026% in gnomAD database and has not been reported in 1000 genomes databases.

**Clinical and Literature evidence:** This variant has been previously classified as likely pathogenic in ClinVar database [3]. This variant has been previously reported in patients affected with growth retardation,

nephrogenic diabetic insipidus and polyuria in compound heterozygous state [4].

**In-silico predictions:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv), LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

**OMIM phenotype:** Bartter syndrome, type 1 (OMIM#601678) is caused by homozygous or compound heterozygous mutation in the *SLC12A1* gene (OMIM\*600839). This disease follows autosomal recessive pattern of inheritance [2].

**Variant classification:** Based on the evidence, this variant is classified as a likely pathogenic variant.

#### **PKHD1L1: c.6176-1G>C**

**Variant summary:** A heterozygous 3' splice site variation in intron 40 of the *PKHD1L1* gene (chr8:g.109450974G>C, NM\_177531.6, Depth: 175x) that affects the in variant GT donor splice site of exon 40 (c.6176-1G>C) was detected.

**Population frequency:** This variant has not been reported in gnomAD database and 1000 genomes database.

**In-silico predictions:** The *in-silico* predictions of the variant are disrupting by GeneSplicer and MaxEntScan. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

**OMIM phenotype:** Deafness, autosomal recessive 124 (OMIM#620794) is caused by homozygous or compound heterozygous mutation in the *PKHD1L1* gene (OMIM\*607843). This disease follows autosomal recessive pattern of inheritance [2].

**Variant classification:** Based on the evidence, this variant is classified as a likely pathogenic variant.

### **Additional Variant(s)**

**List of additional variant of uncertain significant variants identified:**

Disease	Mr.XXX	Mrs.YYY
Fetal akinesia deformation sequence 3 (OMIM#618389) Myasthenic syndrome, congenital, 10 (OMIM#254300)  <b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/347</b> <b>Genetic Prevalence: 1/479512</b>	<b>CARRIER</b> Gene: <i>DOK7</i> Exon 7, c.1417G>A p.Glu473Lys Heterozygous  <b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">534121</a></b>	<b>CARRIER</b> Gene: <i>DOK7</i> Exon 7, c.1417G>A p.Glu473Lys Heterozygous  <b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">534121</a></b>

<p>Cataract 18, autosomal recessive (OMIM#610019)</p> <p><b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/911</b> <b>Genetic Prevalence: 1/ 3316543</b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>FYCO1</i> Exon 16, c.4171G&gt;T p.Val1391Phe Heterozygous</p> <p><b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">2243397</a></b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>FYCO1</i> Exon 16, c.4171G&gt;T p.Val1391Phe Heterozygous</p> <p><b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">2243397</a></b></p>
<p>Spastic paraplegia 15, autosomal recessive (OMIM#270700)</p> <p><b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/772</b> <b>Genetic Prevalence: 1/2382211</b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>ZFYVE26</i> Exon 31, c.5768G&gt;A p.Arg1923Gln Heterozygous</p> <p><b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">579343</a></b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>ZFYVE26</i> Exon 31, c.5768G&gt;A p.Arg1923Gln Heterozygous</p> <p><b>Classification: Uncertain Significance</b> <b>ClinVar ID: <a href="#">579343</a></b></p>
<p>Hydrocephalus, congenital, 2, with or without brain or eye anomalies (OMIM#615219)</p> <p><b>Mode of inheritance: AR</b> <b>Cumulative carrier frequency:1/319</b> <b>Genetic Prevalence: 1/ 406657</b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>MPDZ</i> Exon 7, c.797G&gt;T p.Gly266Val Heterozygous</p> <p><b>Classification: Uncertain Significance</b></p>	<p><b>CARRIER</b></p> <p>Gene: <i>MPDZ</i> Exon 7, c.797G&gt;T p.Gly266Val Heterozygous</p> <p><b>Classification: Uncertain Significance</b></p>

## Recommendations

- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counseling is recommended.

## Methodology

DNA extracted from the blood was used to perform whole exome using a whole exome capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using GenoLab M sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAscope

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has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes(V2), gnomAD (v3.1.2,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

## Sequence data attributes

	Mr.XXX	Mrs.YYY
Total reads generated	15.62Gb	16.93Gb
Data ≥ Q30	85.94%	86.9%

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

## Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.



- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- The variants of uncertain significance and variations with high minor allele frequencies which are likely to be benign will be given upon request.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [5].

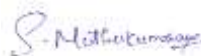
## References

1. Richards, S, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8. PMID: 18842627; PMCID: PMC2686440.
3. <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000242488.8>
4. Wongsasak S, et al. A novel *SLC12A1* gene mutation associated with hyperparathyroidism, hypercalcemia, nephrogenic diabetes insipidus, and nephrocalcinosis in four patients. Bone. 2017 Apr;97:121-125. doi: 10.1016/j.bone.2017.01.011. Epub 2017 Jan 14. PMID: 28095294.
5. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

**This report has been reviewed and approved by:**



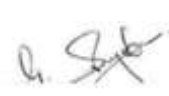
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## APPENDIX: Current gene list used for carrier screening of pathogenic and likely pathogenic variants:

AARS2, ABAT, ABCA1, ABCA3, ABCD4, ABCG5, ABCG8, ACD, ACKR1, ACO2, ACOX2, ACP2, ACP5, ACTA1, ADAMTS10, ADAMTSL4, ADAR, ADAT3, ADCY6, ADD3, ADGRG6, ADGRV1, ADRB2, ADRB3, ADSL, AEBP1, AGBL5, AGPAT2, AGRP, AGXT2, AHY, AHS, AICDA, AIFM1, AIPL1, AK1, AKR1C4, AKR1D1, ALAD, ALAS2, ALDH18A1, ALDH6A1, ALDOA, ALG12, ALG2, ALG3, ALG9, ALOX12B, ALOXE3, ALX3, ALX4, AMBN, AMMECR1, AMN, AMPD2, AMPD3, ANGPTL3, ANO10, ANO5, ANTXR1, AP4B1, AP4E1, AP5Z1, APOC2, APOE, APRT, AQP7, ARFGF2, ARHGDI, ARHGEF6, ARL2B, ARL6, ARL6IP1, ASPM, ATCAY, ATG5, ATP2A1, ATP2B3, ATP6A2, ATP6V1, ATP8A2, ATPAF2, AURKC, AVPR2, B2M, B3GAT3, B4GALNT1, B4GAT1, B9D1, B9D2, BANF1, BBIP1, BBS5, BBS7, BCAP31, BCL10, BGN, BIN1, BLNK, BLOC1S3, BLVRA, BMP1, BMP2, BMPR1B, BPGM, BRAT1, BRF1, BSCL2, BVES, C12orf57, MTRFR, C19orf12, C1QA, C1QB, C1QC, C3, C8A, C8B, C8orf37, CA12, CASA, CA8, CABP2, CABP4, CACNA1D, CACNA1F, CACNA2D4, CAD, CAPN1, CARD11, CARD9, CARTPT, CASP14, CASR, CATSPER1, CAV3, CBX2, CCB1, CCDC103, ODAD1, CCDC115, CCDC22, CCDC28B, CCDC65, CCDC8, CCDC88C, CCNO, CCT5, CD19, CD247, CD27, CD36, CD3D, CD3E, CD3G, CD40, CD46, CD79A, CD79B, CD8A, CDAN1, CDC6, CDH11, CDH3, CDHR1, CDK10, CDK5, CDK5RAP2, CDK6, CDSN, CEBPE, CENPF, CENPJ, CEP104, CEP152, CEP164, CEP19, CEP41, CEP57, CERS3, CFH, CFL2, CHAT, CHIT1, CHKB, CHMP1A, CHRDL1, CHRM3, CHST14, CHST3, CHST6, CHST8, CIB2, CIDEA, CIT, CKAP2L, CLCN1, CLCN4, CLCNKA, CLCNKB, CLDN14, CLDN16, CLIC2, CLMP, CLP1, CLPB, CLPP, CNGB1, CNM2, CNPY3, CNTN1, CNTN2, CNTNAP1, CNTNAP2, COASY, COG4, COG6, COL6A1, COL6A2, COL6A3, COL9A2, COLEC11, COLQ, COQ4, COQ6, COQ7, COQ9, COX10, COX14, COX20, COX4I2, COX6A1, COX6B1, COX8A, CPA6, CPLX1, CPN1, CRADD, CRAT, CRIPT, CRYAB, CRYBB1, CRYBB3, CSF2RB, CSF3R, CSTA, CT C1, CTNNB1, CTPS1, CTS, CTSF, CUBN, CUL7, CWF19L1, CYB5A, CYP24A1, CYP26C1, CYP2C19, CYP2D6, CYP2R1, CYP2U1, CYP4F22, CYP4V2, CYP7B1, DAG1, DARS2, DBH, DCC, DCHS1, DCPS, DCTN1, DCXR, DDHD1, DDHD2, DDOST, DDR2, DDRGK1, DDX11, DDX59, DES, DGKE, DHH, DHODH, DHTKD1, DIS3L2, DLAT, DLEC1, DLG3, DLX5, DNA2, DNAAF1, DNAAF3, DNAH1, DNAH11, DNAJB13, DNAJB2, DNAJC3, DNAJC6, DNAL4, DNASE1L3, DNMT2, DOCK2, DPH1, DPM2, DPYS, DRAM2, DRC1, DSC3, DSE, DSG1, DSG4, DST, DSTYK, DTNBP1, DUOX2, EBP, ECEL1, ECHS1, ECM1, EDARADD, EDC3, EDN1, EGFR, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2S3, EIF4A3, ELAC2, ELMOD3, ELOVL4, ELP2, EMC1, EMG1, EMP2, ENAM, ENO3, ENTPD1, EPCAM, EPG5, EPO, EPS8, EPS8L2, EPX, ERCC1, ERLIN1, ERLIN2, ESR1, ESRRB, EXOSC2, EXOSC8, EXPH5, EXT1, EXT2, EXTL3, F12, F13A1, F13B, F7, FA2H, FADD, FAM20A, FAN1, FANCI, FANCL, FAR1, FARS2, FASLG, FASTKD2, FAT4, FBP1, FBXL4, FBXO7, FCGR2A, FCGR3A, FCN3, FECH, FERMT1, FERMT3, FEZF1, FGF16, FGF20, FGF3, FHL1, FIBP, FIG4, FKBP10, FKBP14, FLAD1, FLI1, FLNB, FLVCR1, FLVCR2, FMO3, FOLR1, FOXI1, FOXRED1, FREM1, FRMPD4, FSHB, FSHR, FTL, FTS1, FUT1, FUT8, FXN, FYCO1, FZD6, G6PC1, GAB1, GAD1, GAN, GATA5, GBA2, GLC, GCNT2, GDAPI, GDF6, GDF9, GEMIN4, GFI1B, GFPT1, GGCX, GHRL, GHSR, GLIS3, GLRA1, GLRB, GLUL, GLYCTK, GM2A, GMPA, 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